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INFLUENCE OF UNRESTRICTED GRAZING ON NORTHERN SALT DESERT PLANT ASSOCIATIONS IN WESTERN UTAH[1]

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AN INTENSIVE STUDY OF RANGE DETERIORATION

The northern salt desert country of western Utah, important as winter range for livestock grazing, is at present characterized by heavy injury to the plant cover, resulting in reduced forage production and a serious replacement of the most valuable forage species by plants largely unpalatable to livestock. This condition was revealed by the results of a recent range survey of the Intermountain Region[3] by the Intermountain Forest and Range Experiment Station. This survey gave unmistakable evidence of considerable deterioration of plant cover and excessive loss of forage values, and indicated that no small part of the deterioration was to be found on the winter ranges.

In view of these findings, it seemed desirable to make an intensive study to ascertain the nature and causes of this condition as a basis for future management of the range. This paper deals with the data obtained in an intensive study of the plant associations on desert range near Milford, Utah (fig. 1), undertaken in 1933 and 1934, and checked in the field in 1935 and 1936. The associations studied are described in the light of their soil and climatic environments, and evidence is set forth relating their condition to the effects of unrestricted grazing.

The evidence of the data presented is substantiated by the almost unanimous assertions of explorers, such as Carte de Escalante (*1, pp. 125–242*),[4] and of old-time residents of this region, all indicating that in the brief span of one generation an unmistakable deterioration has taken place in the quality of forage as well as in the quantity produced. Many stockmen admit forage deterioration on desert ranges, but part of them, at least, hold drought to be the principal cause. This opinion is not borne out by actual drought data taken on desert areas of this region comparable with those on which the present study was pursued. A brief review of these data makes this clear.

Precipitation records for the region from 1897 to 1936 are summarized in figure 2, *C*, combining the records of two stations, Modena and Frisco. Modena lies 35 miles to the south of Pine and Wah Wah Valleys, in which the study was made, and Frisco is a few miles east of Wah Wah Valley. The temperature-precipitation graphs shown for these two stations (fig. 2, *A* and *B*) give evidence of general agreement. In later years, records at three other stations, Milford and Black Rock,

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[3] The States included in the Intermountain Region are Utah, Nevada, southern Idaho, and southwestern Wyoming.

[4] Italic numbers in parentheses refer to Literature Cited, p. 316.

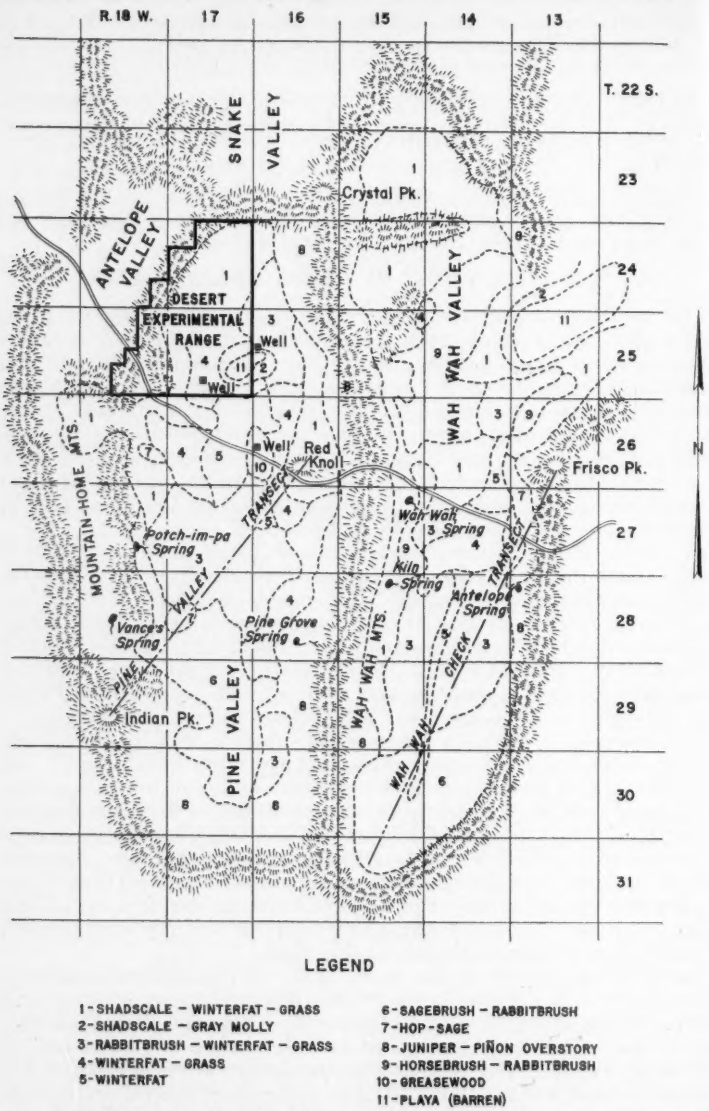

FIGURE 1.—Map of Pine and Wah Wah Valleys and surrounding territory near Milford, Utah, where the present study was pursued, showing forage types and transects run.

east and northeast of Frisco, and Garrison, to the northwest of Antelope Valley, have been in general agreement with those of Modena.

A marked significance in the 1897–1936 precipitation curve in the study of the desert plant associations is in the two drought periods,

1897–1904 and 1928–35. Of these, there seems little question that the former was as long as the other and the more severe. It does not, however, stand out in the memory of ranchers of that day as a period of forage impoverishment. Supplemental feeding of range livestock was unknown at that time; yet fat cattle were obtainable at any season of the year. Fat sheep were marketed on the desert range in midwinter during the decade 1895–1904, according to the testimony of resident stockmen. Such an achievement would have been utterly impossible in the recent drought period, and this very impossibility is eloquent of a radical change in the forage values of the range that is not wholly attributable to drought. The evidence is that in the early days the damaging effect of acute shortage of moisture in drought years was off-

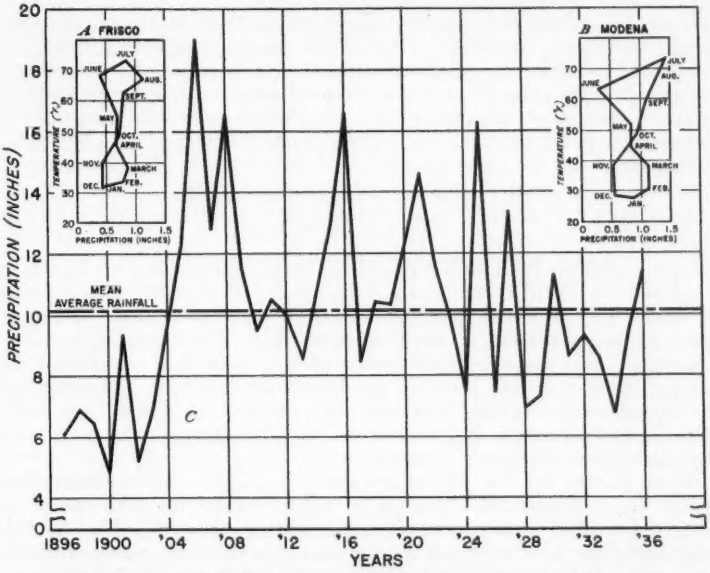

FIGURE 2.—Annual fluctuations in precipitation (*C*) in Pine and Wah Wah Valleys, 1897–1936; derived from records taken at Frisco (1897–1900) and Modena (1901–36). *A* and *B* are temperature-precipitation graphs of the two stations, averaging monthly mean temperatures and total monthly precipitation for the years of record.

set by moderate utilization, which permitted restoration of the normal growth and nutritional function of the range plants.

Drought is not new to the desert valleys of western Utah, but the present disastrous forage impoverishment is of comparatively recent origin. Since drought alone cannot account for the present deterioration in plant communities, nor explain the alarming plant retrogression, logic obviously points to another contributing cause sufficient to upset the vegetational balance, precipitate the downward trend in forage values, and in the two valleys in which the present study was made—subject to the same climatic vicissitudes but to widely different grazing use—to bring about the present great differences in plant mortality and forage volume.

How considerable these differences are may be realized from conditions in these valleys at the end of the recent drought period. The growth increment of all shrubs and grasses in both valleys was negligible in 1934, and in the normal growing season of that year the desert was almost destitute of green. Even the hardiest shrubs lost their leaves in the scorching summer days. Grass nibbled to the earth during the winter of 1933–34, in June, July, and August resembled stubble on badly abused pastures. Soil of thousands of acres of desert land, nearly barren of vegetation, seemed to be lifted away by the wind. Yet with good precipitation in 1935 and 1936, vegetation in the less heavily grazed valley made a comparatively abundant growth, whereas the heavily grazed plants in the other valley failed to respond except in small degree.

Basic facts brought out by this study, indicating the successional trends in desert vegetation, the severe injury from overgrazing to the more desirable forage plants, and their replacement by species of low palatability, should serve as guides to further research as well as in the subsequent administrative control required to bring about successful reproduction of the palatable shrub species and to restore the grasses.

DETAILS OF THE STUDY AREA

LOCATION OF STUDY

Pine and Wah Wah Valleys, which comprise a portion of the desert range near Milford, Utah, were selected for the study because they are characteristic of the drier parts of the Great Basin area. Furthermore, they are similar to each other in size, physiographic features, and plant associations; they support range types that are important throughout the desert area of the Great Basin; they represent distinctly different degrees of grazing use, Wah Wah Valley being the more severely grazed; and also their grazing history is better known than that of other desert areas.

Being nearer to the oldest settlements in the region, Wah Wah Valley has been grazed longer, i. e., for about 70 years as compared with 50 for Pine Valley. In the late eighties sheep were brought in great numbers to Wah Wah Valley and grazed there along with the cattle already present, thereby beginning a long period of double use. Cattlemen in Pine Valley succeeded for about 10 years after this in maintaining a dead-line against sheep. Cattle grazing in the late nineties was probably heavy in Pine Valley, but forage use did not become seriously destructive until about 1910 or possibly 1915. During the last 20 years, or thereabouts, largely through the development of watering places, forage utilization in Pine Valley has exceeded proper use in nearly, though not quite, the same degree that has been common in Wah Wah Valley over a period of 50 years. During the drought period of 1928–35, deterioration of the palatable forage plants became serious enough to be well marked in all parts of Pine Valley.

The climate of this locality is essentially typical of the drier parts of the northern shrub desert. The precipitation of less than 10 inches annually is generally distributed evenly throughout the year, although great fluctuations in the annual and monthly precipitation are characteristic. Potential evaporation, as in all deserts, is high—greater than 60 inches annually, so far as can be judged by records furnished

by the Weather Bureau for Lehi, Utah, situated near the desert edge. The diurnal range of temperatures is great at all seasons, extremes varying from a maximum of more than 100° F. in summer to a minimum of −35° in winter. The sky is so clear and the humidity so low that even in July the nights are cool. The frostless season of 4 months extends from mid-May to mid-September. Winds are fairly frequent and of rather high velocity.

Major desert plant associations are characterized by four general features: (1) Preference for certain soil conditions both of physical nature and saline content; (2) a rather sharp delimitation of the associations; (3) the preponderance of one or two plant species; and (4) the general absence of annuals, except for early spring forms, which in years of ordinary moisture supply are found on the foothills. In contradiction to this generalization is Russian-thistle,[5] an exotic annual that has recently invaded badly deteriorated areas throughout the region.

The position, extent, and number of the various plant associations of Pine and Wah Wah Valleys shown in figure 1 suggest a confusing complexity of vegetation, but there are only two major growth forms, the piñon-juniper of the desert mountain ranges and the short desert-shrub type of the valley plains. The specific associations of the latter present a variety of color and differ in many details, but in their general features they are remarkably uniform in their morphological adaptations to the vicissitudes of the desert environment.

COLLECTION OF DATA

Investigators in the field obtained data on soil, root penetration, and vegetative status of the plant associations. Data on plant associations covered extent and habitat, species composition, degree of injury by grazing use, and the present condition of important species as measured, singly or collectively, by general vigor, presence or absence of young plants, and replacement of palatable by less palatable forage species.

A range survey of the two valleys gave the total number of square miles occupied by each of the 10 plant associations (table 1). Each association, several of which cover from 100 to 600 acres, consists of a mixed plant population of 10 to 20 species of which 2 or 3 are dominant. Within each association are often found plant communities of ¼ to 5 or 6 acres in area (rarely 40 or 50 acres) occupied by one of the major species in nearly pure stand. Soil samples discussed later were taken in these plant communities; the influence of grazing, however, was studied by the status of individual forage plants or groups of plants, sometimes in one association and sometimes in several.

Besides the general survey, detailed studies were made by examining the vegetation along one transect in Pine Valley and another in Wah Wah Valley. In Pine Valley the transect extended a distance of 23 miles from the base of the Wah Wah Mountains in a southwesterly direction across the valley to the summit of Indian Peak (fig. 1). In all, 920 circular point-observation plots (7), each with an area of 200 square feet, were established along the transect at intervals of 132 feet. In Wah Wah Valley, along a similar transect, observations on 180 plots

[5] A list of scientific and common names of the plants discussed in this paper is given on p. 315.

were made. From each plot, data were obtained on vegetation composition, density of cover, and plant mortality.

TABLE 1.—*Areas occupied by various desert plant associations in Pine and Wah Wah Valleys*

Plant association [1]	Pine Valley	Wah Wah Valley	Plant association [1]	Pine Valley	Wah Wah Valley
	Square miles	*Square miles*		*Square miles*	*Sauare miles*
Shadscale—winterfat [2]-grass	115	186	Spiny hop-sage	5	11
Shadscale—gray molly	7	15	Horsebrush — small rabbitbrush		76
Small rabbitbrush — winterfat [1]-grass	105	102	Greasewood	5	
Winterfat [2]—grass	73	22	Juniper—piñon overstory	370	223
Winterfat [2]	25	16	Playa (barren)	3	27
Big sagebrush—small rabbitbrush	75	79	Total area	783	757

[1] For scientific names of plants see p. 315.
[2] Locally known as white sage.

A plant was considered to cover the ground completely in the rare instances when the ground could not be seen through its foliage by an observer standing directly over it. In most cases the value recorded was the fractional spread of the plant when so compressed as to make a complete cover. Thus, if a plant with a total natural spread of 1 square foot made a complete cover of only one-half square foot, it was given a ground-cover (density) value of one-half square foot. When the total ground-cover area for all plants of a given species within a plot totaled less than one-fourth square foot, the presence of the species was recorded merely as a trace. The total area of all vegetation on a 200-square-foot sample plot was determined by adding the individual figures. Density percent was obtained by dividing the total area in square feet by 2.

Mortality data were obtained by counting the number of living and dead plants of each major species on the plots.

In addition to the transect composed of circular plots, small "belt" transects were established near but not on the main transect in each plant association. Care was exercised to locate these at random. On the belt transects vascular ring counts were made of 100 shrubs of each major species in each association from all plants whose crown centers fell within the transect boundaries. Two men, equipped with hand lenses and trained to take the age data, made separate counts of the vascular rings for each stem and compared results. If, owing to the irregularity, incompleteness, or indistinctness of the vascular rings, large discrepancies occurred, recounts were made. In order to check the accuracy of the method, ring counts were made on stems taken from plants on railroad fills and in an abandoned cemetery, where the maximum possible age of the shrubs was known.

The type of root system, the horizontal and vertical spread of the roots, and the general relationship of root penetration to the soil-salt horizon were recorded for each major species. These data were obtained from excavations made in associations dominated by the species.

NATURE OF SOILS AND SOIL REGION

Soil samples were taken in several plant communities in the associations in Pine Valley with the exception of the juniper, which grows in very shallow soil. In all, 14 trenches were dug in the various communities, and profile maps of the soil horizons were drawn. Three to five soil samples were composited from the trenches at each of the following depths in centimeters: 0–15, 15–30, 30–60, and 60–90. Each sample was carefully analyzed for total salts, total organic matter, pH value, total nitrogen, moisture content, moisture equivalent, water-holding capacity, and the percentages of gravel, sand, silt, and clay (table 2).

TABLE 2.—*Soil data* [1] *from the major plant communities indicated, occurring in various associations crossed by the Pine Valley transect*

Plant community within associations	Depth of soil	Average salt content (bridge)	pH value	Total organic matter	Total nitrogen	Moisture equivalent	Water-holding capacity [2]	Soil moisture [3]	Gravel	Sifted sample		
										Sand	Silt	Clay
Grass (blue grama, galleta, sand dropseed).	*Cm.*	*P. p. m.*		*Pct.*	*Pct.*			*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
	0–15	200	8	1.25	0.091	18.1	37	2.2	59	62	29	9
	15–30	210	8	1.29	.102	18.6	47	3.3	50	51	43	6
	30–60	895	8	1.21	.101	20.8	51	2.6	66	59	34	7
	60–90	1,160	8	1.47	.094	20.7	37	2.9	74	57	34	9
Shadscale	0–15	200	8	1.16	.082	17.9	34	1.5	48	64	27	9
	15–30	468	8	.85	.094	18.5	45	2.1	27	53	41	6
	30–60	1,065	8	1.14	.087	24.8	45	2.4	80	64	27	9
	60–90	5,065	8	.69	-------	24.6	54	2.1	72	68	30	2
Winterfat	0–15	290	8	1.29	.087	20.3	54	1.9	32	54	26	20
	15–30	290	8	.63	.058	22.1	46	3.1	1	46	40	14
	30–60	6,015	8	.54	.054	21.0	64	4.9	0	54	44	2
	60–90	3,742	8	.26	.050	13.6	38	4.3	71	72	28	0
Small rabbitbrush	0–15	342	7.5	.80	.058	14.5	30	1.8	23	74	15	11
	15–30	962	8	.56	.046	15.4	35	4.2	18	75	13	12
	30–60	1,358	8	.52	.045	14.2	44	6.7	15	73	14	13
	60–90	8,648	8	.28	.034	30.0	59	8.1	12	47	39	14
Spiny hop-sage, blue grama, galleta.	0–15	244	7.5	.67	.061	11.9	33	2.7	19	92	3	5
	15–30	582	8	.41	.043	12.6	38	4.8	4	89	5	6
	30–60	323	8	.58	.141	8.7	46	4.9	6	93	4	3
	60–90	684	8	.50	.038	11.7	38	5.2	6	93	4	3
Big sagebrush	0–15	200	7.5	1.03	.061	13.5	37	3.2	16	82	15	3
	15–30	302	7.5	.84	.060	15.1	43	4.5	8	78	16	6
	30–60	716	8	.72	.050	11.6	44	5.0	9	76	18	6
	60–90	1,080	8	.21	.027	-------	34	5.0	12	84	16	0

[1] Based on average of 3 or more samples.
[2] Hilgard's method.
[3] July 1934.

The soils of the Pine Valley transect are characterized by five general features: (1) A predominantly sandy nature; (2) a low saline content in the first 30 cm. of soil, regardless of the plant communities; (3) a variable saline content below the 30-cm. level, closely correlated with the type of vegetation; (4) a hydrogen-ion concentration between pH 7.5 and pH 8; and (5) an extremely low content of organic matter and nitrogen.

Physical differences in the soils supporting the various plant communities are slight. The degree and direction of slope, the presence or absence of hardpans, and the variation in the salt content of the subsoil seem to play an important role in the extent and distribution of the desert vegetation. For example, spiny hop-sage

and big sagebrush grow on soils which, in the main, contain little salt (*4*, *6*) to a depth of about 3 feet (90 cm.) or more. The subsoils are more porous, and their water-holding capacity is lower.

All soils in these valleys have a greater amount of gravel at the surface than immediately below; winterfat soils in some cases have 32 times as much. From the condition of vegetative cover in the

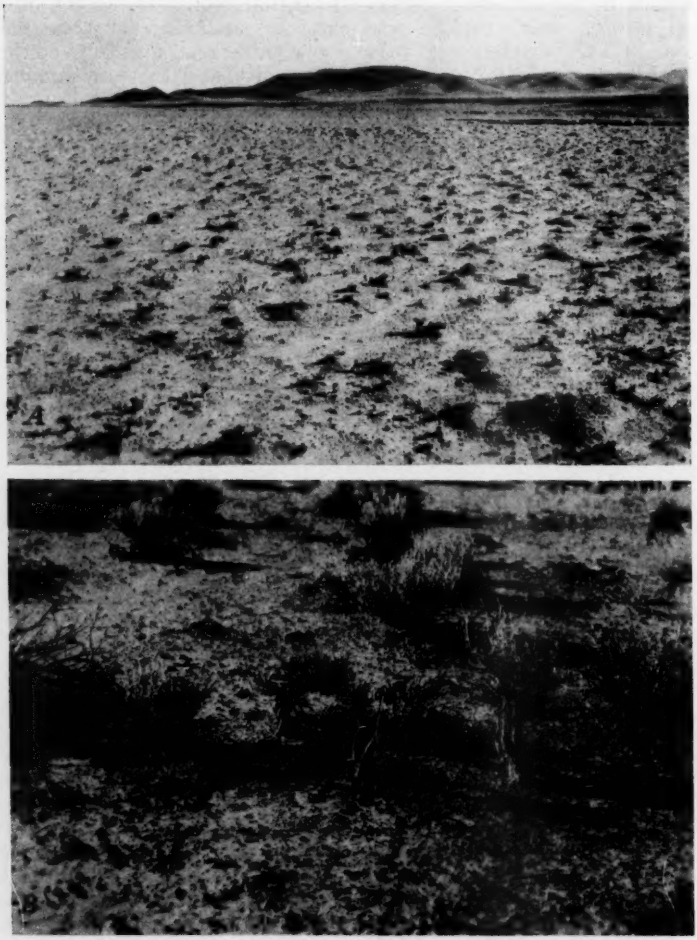

FIGURE 3.—Aspects of erosion in Wah Wah Valley: *A*, General view of a wind-eroded area in the winterfat association; *B*, pedestaled winterfat plants.

various associations it may be deduced that the surface accumulation is not so much an index of percent of gravel in the soil as of the deterioration of the vegetation, which when severe permits rapid wind erosion. The stifling clouds of dust that race over the desert valleys are fed from areas largely denuded of vegetation (fig. 3, *A*).

That a heavy soil movement is occurring on this area is borne out by several observed facts. The pedestaled nature of many desert plants, especially winterfat (fig. 3, *B*), shows that 1 to 6 inches of soil have been removed from below their crowns since their establishment. In

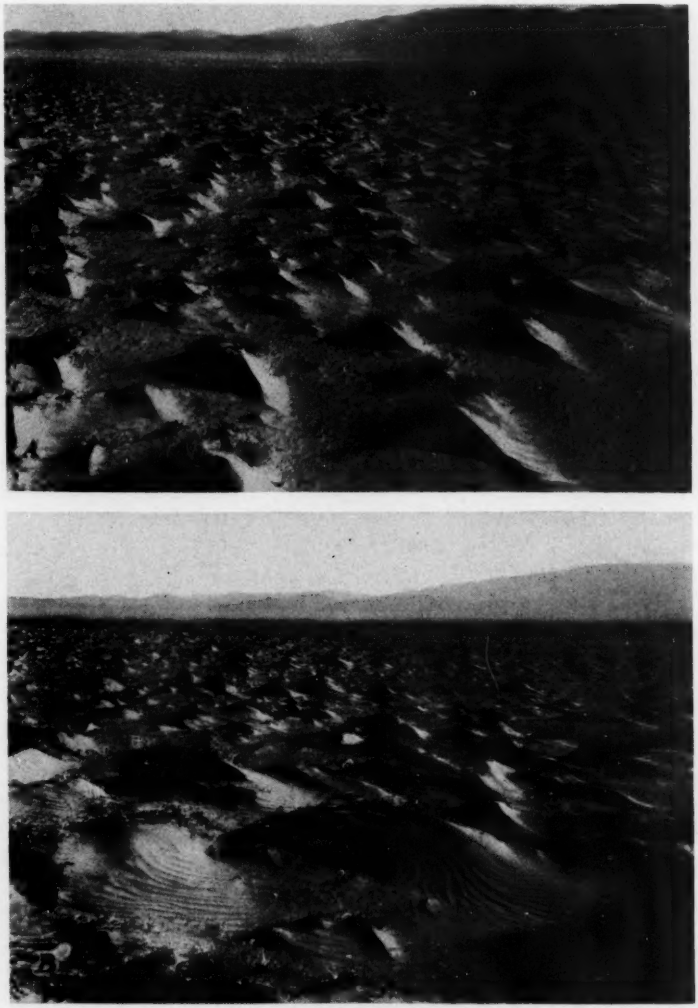

FIGURE 4.—*A* and *B*, Embryonic dunes in Wah Wah Valley, winterfat association.

such cases the height of crown above the soil is not an age factor, for many young plants protrude as much as old ones. Except near the relatively few natural dune areas, pedestaled plants occur only in associations obviously deteriorated through grazing. Many embryonic dunes (fig. 4, *A* and *B*) along highways and on the plant areas

under observation are known to be but a few years old; and a juniper forest on a presettlement dune area in the northeast corner of Pine Valley shows what appears to be a greatly accelerated recent and disastrous dune movement (fig. 5, *A*).

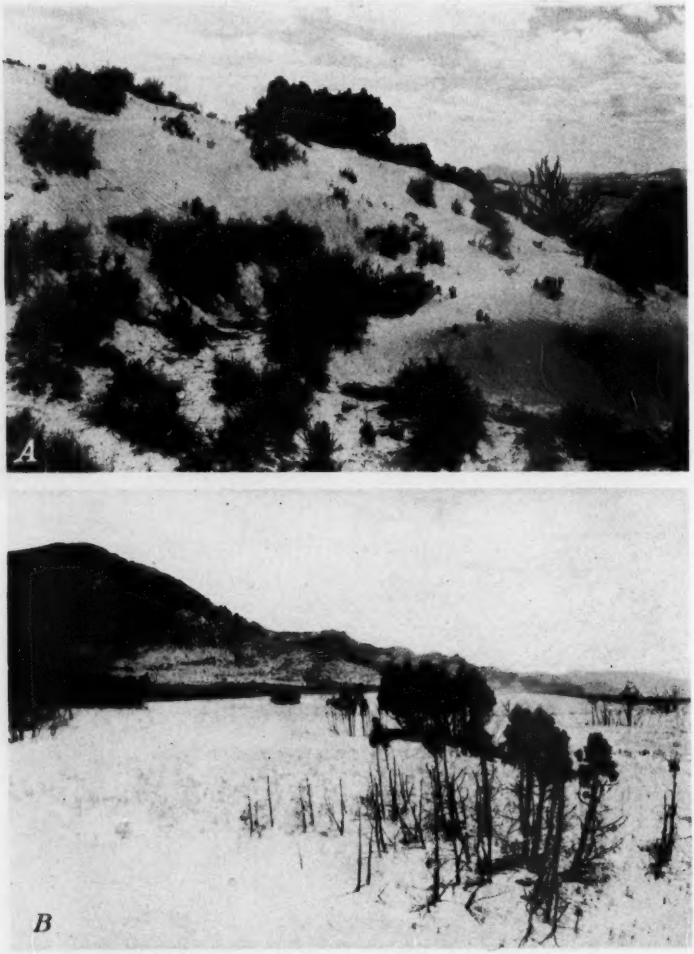

FIGURE 5.—Aspects of erosion in Pine Valley. *A*, Juniper trees buried by sand dune; *B*, an advancing dune in the juniper-piñon association.

Groves of dead junipers newly buried by sand in this old dune area stand as mute evidence of newly aroused activity of dune movement, and living young junipers scattered in this ghost forest, at various distances in the rear of an advancing dune (fig. 5, *B*), furnish evidence as to the average annual dune movement compiled in table 3.

TABLE 3.—*Rate of dune movement in Pine Valley, as estimated from ages of living juniper trees, in rear of advancing dunes*

Tree No.	Distance of tree from dune	Age of tree	Estimated yearly movement
	Feet	*Years*	*Feet*
1	608	25	24.32
2	830	31	26.77
3	881	38	23.18
4	807	40	20.18

ROOT PENETRATION OF PRINCIPAL DESERT PLANTS

The root penetration of desert plants, as shown by the nature and extent of the root systems, is influenced by two major factors, soil salinity and the nature of the plant.

The four grasses, blue grama, galleta, Indian ricegrass, and sand dropseed have root systems the large branches of which are entirely within a shallow soil layer no deeper than 30 to 45 cm. with a salt

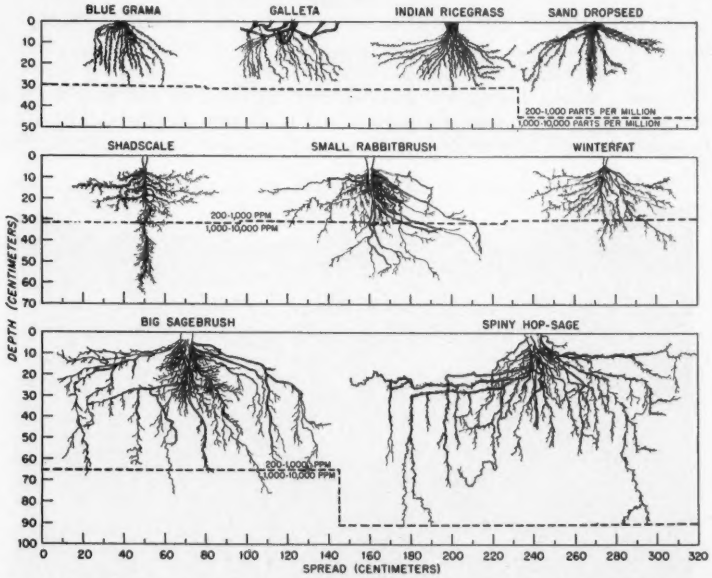

FIGURE 6.—Root penetration of nine principal desert plants, indicating their respective endurance of salt salinity. The heavy broken line marks zones of salt content—above, 200 to 1,000 parts per million; below, 1,000 to 10,000.

content of less than 1,000 p. p. m. (fig. 6). The roots are finely branched and occupy this surface layer rather fully. The roots of shadscale, small rabbitbrush, and winterfat, on the other hand, have some larger roots that penetrate into soil layers having a salt content in excess of 1,000 p. p. m., and also have a developed taproot system. Big sagebrush and spiny hop-sage send down roots to approximately 80 or 90 cm., but the soils supporting these two species are usually

porous and newly deposited alluvial material, frequently in front of a ravine. This is especially true of the hop-sage, which was always found near the base of an alluvial cone where storm floodwaters spread out and afforded irrigation. Although sagebrush often sent a few roots into a soil layer of greater salinity, in no case were hop-sage roots found growing in soil that had a salt content of more than 1,000 p. p. m.

Growing only in deep alluvial soils with the hardpan 3 to 5 feet below the surface, the roots of hop-sage and sagebrush offer less competition to grasses in the associations dominated by these two plants than is encountered from shrubs in other associations, such as shadscale, rabbitbrush, and winterfat. Since these last-named species usually grow on soils having a depth of only 12 to 24 inches above hardpan, most of their roots occupy the same soil layer as those of the grasses and therefore compete directly with them for moisture. Both historical and ecological evidence point clearly to the greater previous abundance of valuable grasses in the sagebrush and hop-sage associations.

EVIDENCE OF DETERIORATION IN PLANT ASSOCIATIONS

DESERT GRASSES

Grasses, which today are insignificant in most of the desert-shrub associations, were originally important members of practically all of them (*2, 5, 6*). Old residents maintain that desert vegetation formerly

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FIGURE 7.—A good stand of winterfat in a lightly grazed valley in Nevada.

consisted of mixed shrub-grass associations, except in the low, broad watercourses along the valley edges where winterfat (white sage) existed in almost pure stands. (Fig. 7.) They say also that when grazing began this species was the principal shrub of the valleys, and that big sagebrush and black sagebrush (black sage), with abundant grasses interspersed, dominated the foothills.

It is probable that grasses never existed on the desert in pure stands of any great area, but were always associated with shrubs. At present they are most abundant on the foothills in a narrow belt lying between the shadscale association in the valley and the juniper association at the higher elevations. Shadscale is now the most important shrub component of the grass communities. Grass species, in order of their abundance in the Pine Valley transect, are blue grama, sand dropseed, galleta, and Indian ricegrass (*3*). The first three of these grasses, usually combined but sometimes occurring separately, frequently form almost pure communities over small tracts of less than an acre within the associations. This probably is to be explained rather by priority of occurrence than by reaction to peculiar soil conditions. In all grass communities, the sites have a gentle slope with good drainage, but

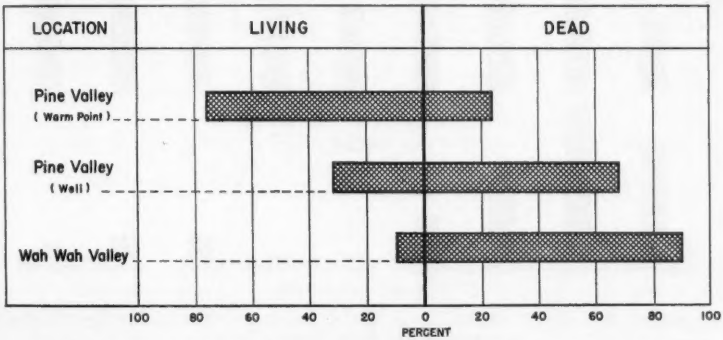

FIGURE 8.—Percent of Indian ricegrass plants alive and dead in three significant locations, the moderately used area of Warm Point, the now heavily used area adjacent to a well, and the long-used, severely grazed Wah Wah Valley.

in many cases the saline content of the soils below 30 cm. is high. At this depth, or slightly below it, a calcareous layer commonly exists, probably at the average depth of moisture penetration.

Indian ricegrass, probably the most conspicuous if not the most abundant grass on the desert in 1880, is now reduced to a very low density under the longer and more severe grazing practice in Wah Wah Valley. It readily adapts itself to many habitats, although it seems to be best adapted to sandy soils. It is significant that this most palatable of all desert grasses is threatened with almost complete extinction. That its disappearance on large areas of the desert is traceable directly to grazing is illustrated in figure 8. On three sites, comparable for Indian ricegrass production, except for their grazing history, widely different mortality conditions exist. At Warm Point, 10 miles from permanent water, there are four times as many living plants of this grass as in the vicinity of a well drilled 10 years ago, where water is always available to livestock. Near the well, more than half the still visible plants are dead as the result of overgrazing, trampling, and congestion of the livestock around water.

The total plant cover in those communities of Pine Valley (fig. 9) in which blue grama is dominant exceeds per unit area that in all other communities save sagebrush and juniper-piñon. The desert grasses, however, have a high mortality rate and are low in vigor from continued close cropping for the past 30-odd years.

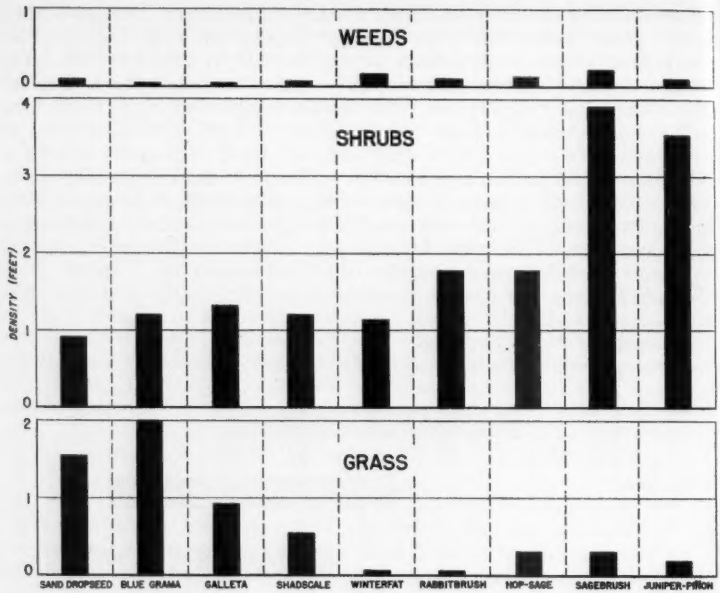

FIGURE 9.—Density of vegetation of shrubs, grasses, and weeds in the principal communities in Pine Valley, Utah.

Palatability of desert plants, besides indicating the relative extent to which they have been grazed in the past, affords a key to the interpretation of the successional trends of desert vegetation as revealed in this study. Under unrestricted grazing the more palatable plants such as Indian ricegrass, spiny hop-sage, and black sagebrush are seriously reduced, whereas the less palatable species such as small rabbitbrush, often untouched, have increased greatly in recent years. The data given in the following tabulation, from unpublished records of the Intermountain Forest and Range Experiment Station, represent the palatability of the current year's growth of the various species, or the percent that, on properly grazed ranges, is likely to be utilized by sheep and cattle during winter grazing:

	Palatability Percent
Grasses:	
Indian ricegrass	85
Sand dropseed	40
Galleta	60
Blue grama	40
Weeds:	
Russian-thistle	25
Shrubs:	
Spiny hop-sage	80
Black sagebrush	80
Winterfat	60
Shadscale	40
Small rabbitbrush	20
Big sagebrush	20

Desert grasses subjected to heavy grazing have reproduced very poorly during recent years. The age of grasses cannot be obtained with assurance since they do not have annual rings. It is thought, however, that with such grasses as blue grama, which in these high desert valleys increase the size of their clumps slowly, a rough separation can be made between old and young plants. Dropseed grass clumps also increase in size very slowly and somewhat regularly. Near absence of small plants in both valleys indicates an extremely low reproduction of these grass species during the last several years where the grazing was severe, whereas on a few lightly grazed tracts 3 or 4 miles from the transects young plants are fairly common.

On comparatively large areas in several associations of Wah Wah Valley galleta grass is now rather abundant, having occupied sites formerly producing abundant ricegrass. Numerous dead clumps of ricegrass are found on some sites now occupied by galleta, squirreltail, and Reverchon three-awn, galleta usually constituting most of the grass cover. This prevalence of galleta on severely grazed ranges is probably owing to its usual low height in the cool weather of fall, winter, and spring, which affords it some protection against full utilization, especially when a light fall of snow may cover it almost entirely. Since the open, irregular growth habits of galleta makes any estimate of its age highly uncertain, no measurements were attempted.

SHADSCALE

Shadscale dominates more square miles of valley desert than any other plant species and in Pine and Wah Wah Valleys occupies more than one-fourth of the total area below the juniper belt (fig. 10). Al-

FIGURE 10.—Shadscale association on Pine Valley transect, with Indian Peak in the distance.

kali-tolerant if not alkali-requiring (*4*, *6*), it is found in two distinct soil habitats—the foothills where grass is an important member of the association, and the low, flat soils abutting the barren playa bottom where gray molly, a pronounced halophyte, is the chief commensal. On upland shadscale soils, hardpans rich in salt are found at 1 to 2

feet below the surface, whereas in the playa bottoms a high salt content is characteristic even of the surface soil.

Shadscale of the upland type has shown a mortality of approximately 30 percent during the drought period on both protected and unprotected areas, the heaviest losses occurring on its upper edge in the mixed shadscale-winterfat-grass association. However, this high mortality does not occur on the playa bottoms where the species is more vigorous. Since high mortality of upland shadscale followed the drought of 1931, it seems probable that this plant is better adapted to endure physiologically rather than physically dry soils, and that its preferred habitat is the heavier soil of the bottom lands with higher moisture-retaining properties.

Old residents of the desert maintain that on the upland habitats shadscale has increased both in acreage and in volume at the expense of winterfat and grass. Its competition with grass was perhaps greatest near the upper margins and with winterfat at the lower extensions of the association. No statistical information is available on its total volume on these upland habitats 50 years ago, but an analysis of the age[6] and mortality data for the various components of the association indicates the extension of shadscale on both margins, as shown in table 4.

TABLE 4.—*Percentage of shadscale and winterfat plants, in the upper and lower transition margins of a shadscale association, having 0–20 and more than 40 annual rings*

Location and number of annual rings	Shadscale	Winterfat
Upper margin:	*Percent*	*Percent*
0–20	40	8
40	38	68
Lower margin:		
0–20	22	10
40	36	49

In view of the relatively low palatability of shadscale and the high palatability of winterfat, the data from their mixed association seem to warrant the following conclusions:

(1) On the average, shadscale is now suffering some loss of vigor from heavy grazing, as is shown by the fact that 50 plants (38 live ones) per plot have a ground cover of 3.2 percent in Pine Valley, in comparison with 44 plants (29 live ones) per plot and a ground cover of 1.6 percent in Wah Wah Valley. The high mortality of shadscale in all parts of the desert and equal occurrence of mortality on grazed and protected range, indicates low hardiness, against drought at least, on all sites.

(2) The ground cover of 10 winterfat plants in Pine Valley has been conservatively estimated at 1 square foot, whereas 59 plants per plot in Wah Wah Valley produced merely a trace, emphasizing the impoverishment of this species under prolonged heavy grazing. In Pine

[6] It is recognized that, because winterfat, shadscale, and spiny hop-sage, which belong to the Chenopodiaceae, may under peculiar conditions lay down more than one vascular ring in a year, the total number of vascular rings may perhaps indicate only the approximate age, or somewhat more than the real age. In an abandoned desert cemetery, the oldest chenopods growing on graves dated 30 to 50 years ago failed to show any plant with a number of rings that exceeded the number of years since burial. Excess of ring counts over age in years does not therefore seem to be great for the period being considered. Since in nearly all cases, the ring counts are relative between the different species or between different samples of the same species during the same years, comparisons seem valid.

Valley 24 percent, and in Wah Wah Valley 55 percent, of winterfat plants in the shadscale-winterfat-grass association are dead (fig. 11) as the result of unregulated use.

(3) Similarity of the ring counts of the two shrub species in the well-established mixed associations indicates that they were associated during the early days of grazing on the desert. Thirty-six percent of the present shadscale plants, and 49 percent of the winterfat plants have more than 40 annual rings. That shadscale is invading winterfat communities is shown by the fact that 22 percent of the former in the transition zone has less than 21 rings, while only 10 percent of the latter falls in this class. Both the spines and the fact that the palatable leaf growth strips off leaving the unpalatable stems protect shadscale to some extent against excessive grazing.

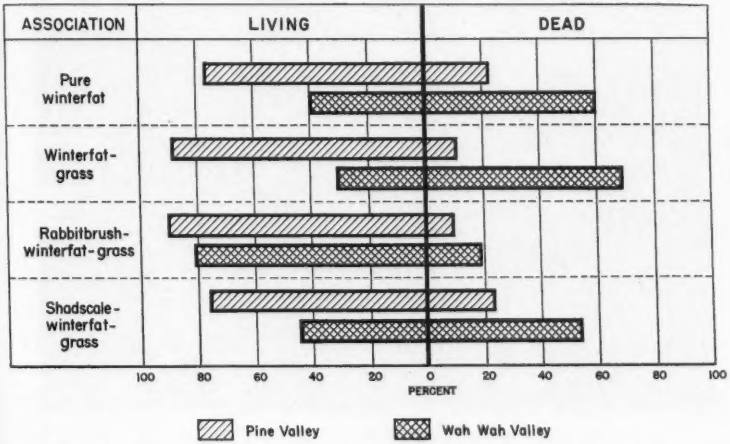

FIGURE 11.—Percentages of living and dead winterfat plants in the plant associations indicated in Pine and Wah Wah Valleys.

WINTERFAT-RABBITBRUSH

Winterfat and small rabbitbrush are considered together because records from old residents supported by an abundance of ecological data show that the desert valleys during the early history of grazing were dominated by the winterfat-grass association; the hundreds of square miles of small rabbitbrush today is a product of the present century; and this species is establishing itself rapidly on sites from which winterfat is disappearing at an alarming rate (fig. 12).

Winterfat reaches its best expression in almost pure stands on broad, flat watercourses of the valleys, where the soil is somewhat better watered by accumulations from the melting snows of spring or torrential downpours of summer rain. In addition, as one component of a mixed stand, it extends normally in all directions up the gentle valley slopes to the mixed shadscale associations.

Rabbitbrush invasion has been most active on the more sandy soils and least active on the naturally irrigated lands of the watercourses. Competition between the two species for possession of the desert

has been severe (*8*), as is shown by the present sharp lines which separate them and the almost total dominance of each in its own territory. Small gray circular islands of winterfat in green stretches of rabbitbrush or a wedge of the latter indenting the margin of an expanse of winterfat are characteristic features of these plant types. This situation is perhaps explained by slight physical and chemical soil differences, although hardpans and a high saline content of the subsoil characterize both conditions.

The rapid increase of young small rabbitbrush plants and the high mortality of palatable forage species in Wah Wah Valley establish grazing as a major cause of deterioration in the vegetation. In view of the great difference in the palatability of the two species, together

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FIGURE. 12.—Rabbitbrush (dark-colored shrubs) invading weakened winterfat areas in Antelope Valley not far from Pine Valley transect.

with the difference in intensity of grazing use between Pine and Wah Wah Valleys, the following facts gained from the study are significant:

(1) The mortality of small rabbitbrush in both valleys was less than 10 percent, but that of winterfat was 23 percent in pure winterfat communities in Pine Valley, 59 percent in Wah Wah Valley, and even higher in the winterfat-grass association (fig. 11).

(2) In Pine Valley 30 percent of the rabbitbrush, and in Wah Wah Valley 43 percent, were produced during the decade 1927–36. The same period accounted for 1 percent of the winterfat in Pine Valley, and none on the sites studied in Wah Wah Valley (fig. 13).

(3) In pure stands of winterfat in Pine and Wah Wah Valleys only 1 percent of the plants had less than 10 vascular rings, whereas in pure stands in Snake Valley about 25 miles to the north, where grazing has been much lighter than in either of the other valleys, 8 percent were in this class (fig. 14).

(4) On the 200 square miles or so occupied by these plants growing together in association in Pine and Wah Wah Valleys there is an average of one winterfat plant to the square foot. Of these winterfat

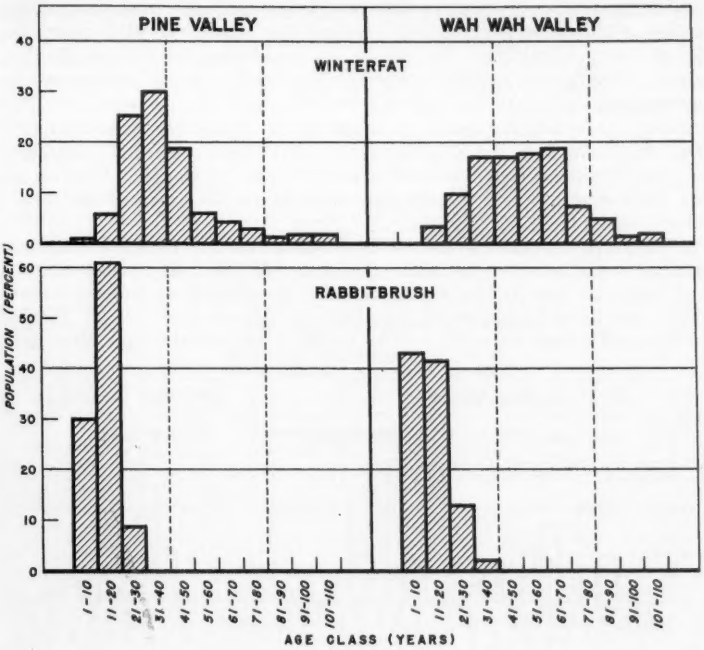

FIGURE 13.—Winterfat and rabbitbrush plants in mixed association, classified by vascular rings, Pine and Wah Wah Valleys.

plants, 10 percent in Pine Valley and 19 percent in Wah Wah Valley are dead (fig.11). The live plants furnish a ground cover of 1.5 percent of a total ground cover of 1.7 percent in Pine Valley and of 0.09 percent of 0.36 percent in Wah Wah Valley.

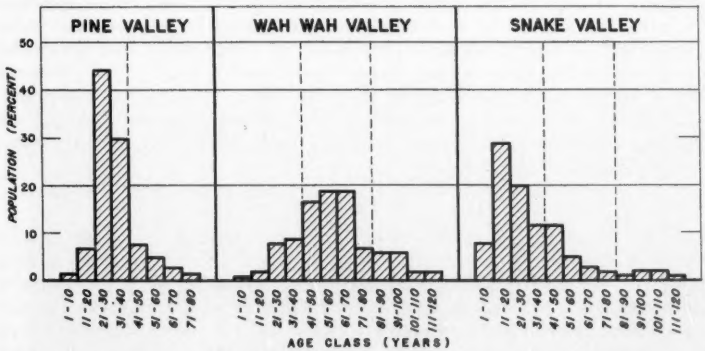

FIGURE 14.—Winterfat plants, classified by the number of vascular rings, form nearly pure populations in Pine, Wah Wah, and Snake Valleys. Young plants are most abundant in Snake Valley where the scarcity of watering places limits grazing to the periods when snow is available for stock water.

(5) The lowest mortality for winterfat in both valleys is found in the rabbitbrush communities where the winterfat plants are few. The low palatability of the dominant shrubs makes forage cover as a whole relatively unpalatable to livestock. It is therefore not heavily grazed.

Small rabbitbrush plants in these plant types are predominantly 20 years old or less (fig.13), indicating a recent invasion. The shrub is normally long-lived, however, as evidenced by the fact that on one area 26 percent of the plants are more than 30 years of age and 4 percent more than 40 (fig.15).

A puzzling feature of the rabbitbrush invasion of winterfat associations is its apparent arrest. In spite of the 100 square miles of land open to the invasion because of the depletion of the natural cover, few invading young rabbitbrush plants are found beyond sharply delimited outposts. The explanation of this paradox prob-

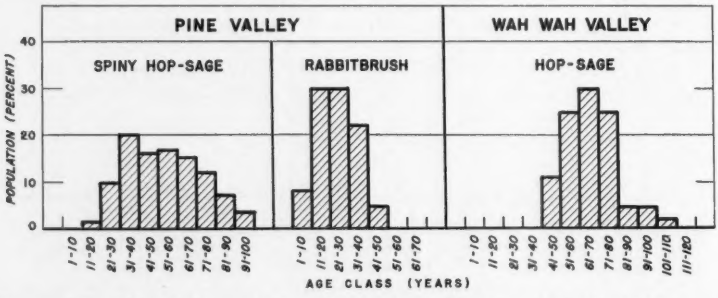

FIGURE 15.—Spiny hop-sage and small rabbitbrush on favorable sites in Pine and Wah Wah Valleys, classified by the number of vascular rings.

ably lies in the present widespread and dense occupation by Russian-thistle, which within the last 15 years has overrun all badly deteriorated areas.

Russian-thistle is both a blessing and a curse to the desert; its seeds germinate at any period of the growing season when moisture is available, and the plants in a short time afford protection against wind erosion; but on the other hand, seedlings of more palatable perennial species under heavy grazing appear practically unable to compete with it. It is a hardy invader in good years, but like all annuals, native or exotic, cannot maintain a plant cover in years of severe drought. In Wah Wah Valley it varied from 20 percent of the ground cover in 1932 to practically nothing in 1934.

HOP-SAGE

One of the most palatable desert shrubs is spiny hop-sage. The root chart (fig. 6) and table 2 show this species to be definitely restricted in habitat; its total area on the desert was never large. The soils in which it thrives are the most free from salt of those supporting any major association. Its preferred habitat seems to be in sands free of hardpans, often granitic, and occasionally watered by intermittent streams.

Approximately 85 percent of the plants in what was classed as pure hop-sage stands in Wah Wah Valley are dead (fig. 16), and the remain-

der nearly so. The number of vigorous plants throughout most of the open winter range has been greatly reduced by overgrazing. Under lighter forage use in Pine Valley, 37 percent of the plants in pure stand are dead and the others severely injured. Since no reproduction whatever was found to have occurred during the last 10 years

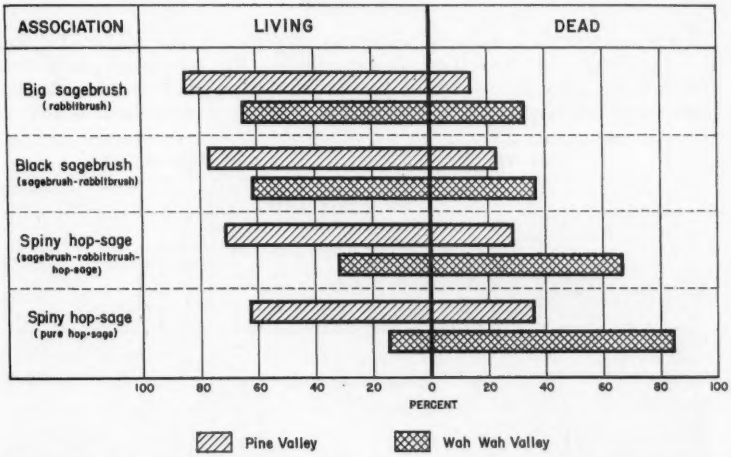

FIGURE 16.—Percentages of living and dead plants of big sagebrush, black sagebrush, and spiny hop-sage in various plant associations in Pine and Wah Wah Valleys.

(fig. 15) and but 1 percent during the last 20 years, the conclusion is that the species cannot withstand even moderately heavy use and that the early extermination of this plant is almost certain. Numerous dead and dying plants of blue grama and galleta bear witness that the hop-sage association, once rich in both quality and quantity of forage, is now on the verge of total extinction (fig. 16).

FIGURE 17.—A hop-sage association in Pine Valley, showing the hedged shrubs and stubble of grama grass.

Small rabbitbrush is invading the hop-sage association and will in all probability, under present grazing practices soon succeed that species on many tracts. On an area used for continuous bedding of sheep in a hop-sage association in Pine Valley, rabbitbrush has supplanted all of the original palatable vegetation.

SAGEBRUSH

Two sagebrushes, notably different in their choice of habitat and in forage value, occupy these desert lands. Big sagebrush, relatively unpalatable and little grazed except under conditions of cold and deep snow or where other forages are lacking, is confined largely to

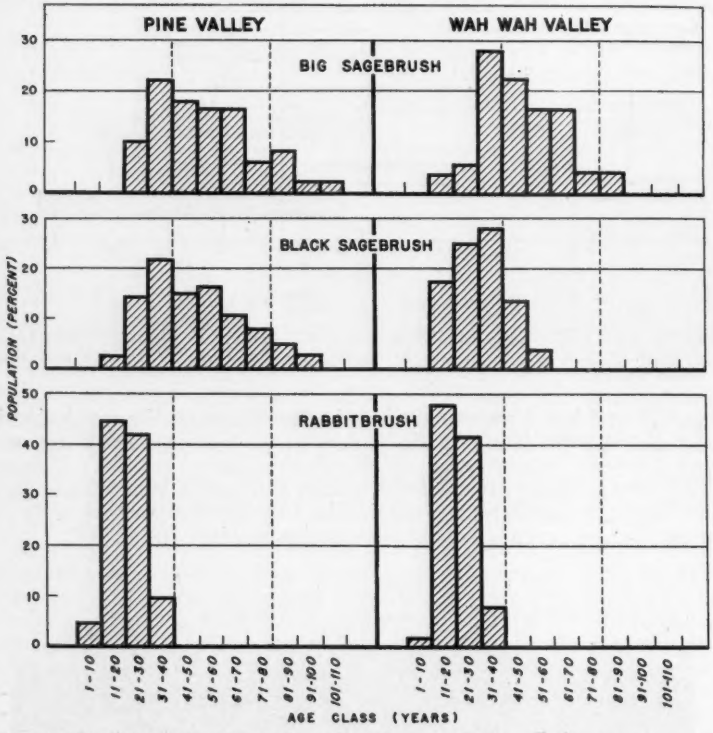

FIGURE 18.—Big sagebrush, black sagebrush, and rabbitbrush growing in association in Pine and Wah Wah Valleys, classified according to the number of vascular rings.

the deep porous soil of the valley uplands, where it makes up the greatest density of plant cover of any association on the desert. The highly palatable black sagebrush constitutes the chief understory of the juniper-piñon association of the hillsides and is also abundant along sides of shallow drainage channels at the upper valley edges. Both sagebrushes grow best on soils free from salt, at least to the ordinary feeding depth of the roots. Usually, black sagebrush occurs on

shallow, rocky soil in contrast with the arable, porous soil preferred by big sagebrush.

Taxonomically, these two sagebrushes are difficult to distinguish. The most constant difference is the light-colored flower stalk and the numerous heads of the large species, as contrasted with the brownish flower stalk and few heads of the smaller. Sheep, however have little difficulty in distinguishing them; black sagebrush is almost invariably closely browsed, while big sagebrush, even when immediately adjacent, often remains almost untouched.

A comparison of the mortality records for the two species in both Pine and Wah Wah Valleys is given in figure 16. Two significant facts are apparent: (1) Mortality is lower for both in the less heavily

FIGURE 19.—Big sagebrush reduced in size by grazing and invading rabbitbrush on a bed ground in Wah Wah Valley.

grazed Pine Valley than in Wah Wah Valley; and (2) the mortality of the more palatable black sagebrush is somewhat greater in both valleys.

Stockmen who have operated sheep outfits on the desert for 20 years or more declare that black sagebrush, the most palatable of all desert shrubs with the possible exception of spiny hop-sage, has decreased tremendously. The principal remnant of any consequence in these two valleys is in the juniper belt, where the plant growth is ordinarily covered with snow in midwinter and is thus protected for a season from excessive grazing.

Small rabbitbrush is, in volume, an important component of these plant types. The age-class data (fig. 18) reveal it as an invading species, just as in other associations important to desert grazing, and indicate that here, too, this invader of low forage value is taking possession of immense areas that formerly supported palatable shrubs (fig. 19).

Big sagebrush communities are chosen more frequently than black sagebrush as bedding grounds for sheep inasmuch as big sagebrush occupies the uplands which are preferred for this purpose. They are

in this way exposed to longer periods of grazing, and so include even fewer plants under 20 years of age (fig. 18). The species is reproducing in some cases, however, owing to the favorable environmental factors. It has replaced the more palatable winterfat in one large part of Wah Wah Valley. A rather extensive young stand was found also in Pine Valley.

HORSEBRUSH AND GREASEWOOD

Horsebrush occurs in small areas in both valleys, as does greasewood. Horsebrush species are highly unpalatable and harmful to sheep, but because the plants of this genus are eaten under pressure of great hunger they deserve critical study. Most of them occur on shallow upland soils otherwise similar to the shadscale-grass habitats. Many dead Indian ricegrass clumps in the association suggest that horsebrush, like small rabbitbrush, is an invading species. Greasewood, because it occurs only on saline soils with high water tables, is of little consequence in Pine and Wah Wah Valleys.

OPEN FORESTS

The Pine Valley transect passes from the desert-shrub through a juniper-piñon belt, at the upper limits of which it traverses dense stands of singleleaf piñon. At higher elevations it passes through a white fir-aspen association.

Piñon and juniper are overstory members of a single association. The piñon dominates the higher elevations, but at the lower limits of

FIGURE 20.—Juniper in Wah Wah Valley, the lower branches of which have been nibbled by sheep. Note the remains of sagebrush and the invasion of rabbitbrush.

the association the juniper occurs in nearly pure stands which ultimately give way to the valley shrub types (fig. 20). Midway, both occur as commensals in a mixed overstory. Sagebrush species are the principal understory shrubs, with big sagebrush occupying the alluvial

deeper soils and black sagebrush dominating shallow, rocky ones. Other shrubs encountered frequently in this association include serviceberry, dwarf goldenbush, curlleaf mountain-mahogany, green ephedra, broom snakeweed, globe cactus, and bitterbrush.

Grasses, probably abundant in the juniper-piñon association before the days of excessive grazing, still present a great assortment of species. Of these, the most important are bluebunch wheatgrass, red threeawn, blue grama, blue wild-rye, galleta, Indian ricegrass, squirreltail, and needle-and-thread grasses.

In contrast with the purely desert types, the juniper-piñon association exhibits a striking seasonal periodicity of its flora. In favorable years, a large assortment of early spring annuals are supported on the foothills, followed in turn by summer and fall herbaceous forms. Principal among this weed flora are rockcresses, sandworts, aster, astragalus, wild-cabbage, Douglas morningbrides, hedgehog cactus, wild-daisy, eriogonum, slender buckwheatbrush, gilias (including tin piute), globe cactus, pricklypear, pentstemons, phlox, groundsels, rock goldenrod, and globemallows.

Ponderosa pine is not common to desert mountains, but local stands exist in a few places. The species is found normally at elevations above the piñon-juniper belt and, as isolated communities, occurs at about 9,000 feet elevation all along the Wah Wah range. Associated with it are Douglas fir, white fir, and aspen. The summit of Indian Peak where the Pine Valley transect terminates is for the most part covered with stony talus and consequently the slopes are generally barren. No ponderosa pine occurs there, but white fir, Douglas fir, and aspen grow in sheltered places.

SUMMARY AND CONCLUSIONS

The vegetation in Wah Wah Valley, Utah, which has been grazed for about 70 years, and severely so for about 50 years, is compared with that in the nearby Pine Valley where, owing to the scarcity of stock water, grazing use was heavy only when snow was available until about 20 years ago, when watering places were artificially developed. The plant cover of these two neighboring valleys, comparable in size, in physiographic features, in soils, and in forage-plant associations, differed mainly as a result of the duration and intensity of livestock grazing to which they had been subjected. In order to compare the effects on vegetation of these two types of grazing use, the density, kind, and condition of the forage plants in the two valleys as well as the prevalent soil conditions, were studied on a long transect across each valley.

Palatable perennial grasses, formerly conspicous in all the desert-shrub associations, today are few in number and low in vigor where the grazing has been severe during the protracted period of approximately 50 years. In comparison with those of low palatability, species of high palatability have suffered (1) a greater loss in density of plant cover, (2) a higher plant mortality, (3) a greater decrease in reproduction, and (4) a sharper decline in general vigor.

Palatable shrubs such as winterfat, shadscale, spiny hop-sage, and black sagebrush have also suffered in the same respects as have the grasses under prolonged heavy grazing. Of the forage shrubs, on which ring counts were made, very few plants were less than about 20 years of age. Grasses also showed low reproduction during a long period of years, although the length of the period was not susceptible to accurate determination.

The root systems of the desert forage species studied were found to be largely limited to the upper 30 cm. of soil where the salt content rarely exceeds 1,000 parts per million. The root systems of big sagebrush and spiny hop-sage extend much deeper than this. Associations in which these species are dominant are limited to areas where even the subsoils have a low salt content.

The naturally sparse plant cover when thinned and weakened by unrestricted grazing has permitted heavy wind erosion, and on a few of the worst areas, the beginning of dune formation.

Small rabbitbrush, low in palatability to livestock and once rare on the desert, has on heavily grazed areas replaced to an alarming extent the more palatable species, but is still rare where the palatable species are vigorous.

Russian-thistle, introduced to the desert two decades ago, is not a serious competitor in vigorous shrub communities, but on heavily grazed areas depleted of vegetation it has taken possession and apparently retards reproduction of desert shrubs.

Impoverishment of desert forage, attributed by many to the drought of 1928–35, was not so markedly apparent after the drought of 1897–1904, which was of as long duration and, measured in total precipitation, more severe. The evidence gained from the study of these plant associations does not support the theory that drought is the sole or even the chief cause of present deterioration and depletion of the range. Instead, it points unmistakably to unrestricted grazing as the chief cause of loss of grazing values, invasion of inferior species, and the gradual crowding out of the most palatable range plants.

The data show clearly that the heavy utilization of the forage by livestock must be relaxed in order to provide for restoration of the range to normal producing power and for its subsequent maintenance. The original presence of accessible stock water in Wah Wah Valley permitted heavy range use in the fall and in the spring as well as during winter. The artificial development about 20 years ago of stock water in Pine Valley, formerly grazed only when snow was available, has permitted heavy use in fall and spring as well as in winter since that time. The result during the last few years has been to set in operation on the forage plants the deteriorating forces of overuse.

Permanent and vigorous forage production on the winter ranges will require the sort of range management that avoids complete utilization of the current year's growth and that will give some relaxation in the degree of use during fall and spring. Development of wells and other stock-watering places needs to be accompanied by control and management of livestock lest the new watering places become centers of yet further forage deterioration.

COMMON AND SCIENTIFIC NAMES OF PLANTS DISCUSSED

Aspen	*Populus tremuloides aurea*
Aster	*Aster engelmanni*
Big sagebrush	*Artemisia tridentata*
Bitterbrush	*Purshia tridentata*
Black sagebrush (black sage)	*Artemisia nova*
Bluebunch wheatgrass	*Agropyron spicatum*
Blue grama	*Bouteloua gracilis*
Blue wild-rye	*Elymus glaucus*
Broom snakeweed	*Guiterrezia sarothrae*
Curlleaf mountain-mahogany	*Cercocarpus ledifolius*
Douglas fir	*Pseudotsuga taxifolia*
Douglas morningbrides	*Chaenactis douglasii*
Dwarf goldenbush	*Aploppapus nanus.*
Eriogonum	*Eriogonum heracleoides* and *E. racemosum*
Galleta	*Hilaria jamesii*
Gilias	*Gilia* spp.
Globe cactus	*Pediocactus simpsonii*
Globemallow	*Sphaeralcea caespitosa, S. coccinea,* and *S. grossulariaefolia*
Goldenweed	*Aplopappus falcatus*
Gray molly	*Kochia vestita*
Greasewood	*Sarcobatus vermiculatus*
Green ephedra	*Ephedra viridis*
Groundsels	*Senecio* spp.
Hedgehog cactus	*Echinocereus mojavensis*
Horsebrush	*Tetradymia* spp.
Indian ricegrass	*Oryzopsis hymenoides.*
Jointfir. (*See* Green ephedra.)	
Needle-and-thread	*Stipa comata*
Oreocarya	*Oreocarya confertifolia* and *O. depressa*
Pentstemon	*Pentstemon palmeri* and other species
Phlox	*Phlox longifolia* and other species
Ponderosa pine	*Pinus ponderosa*
Pricklypear	*Opuntia* spp.
Red three-awn	*Aristida longiseta*
Reverchon three-awn	*Aristida glauca*
Rockcress	*Arabis* spp.
Rock goldenrod	*Solidago petradoria*
Russian-thistle	*Salsola pestifer*
Sand dropseed	*Sporobolus cryptandrus*
Sandwort	*Arenaria* spp.
Serviceberry	*Amelanchier alnifolia*
Shadscale	*Atriplex confertifolia*
Singleleaf piñon (piñon pine)	*Pinus monophylla*
Slender buckwheatbrush	*Eriogonum microthecum*
Small rabbitbrush	*Chrysothamnus stenophyllus*
Small sagebrush. (*See* Black sagebrush.)	
Spiny hop-sage	*Grayia spinosa*
Squirreltail	*Sitanion hystrix*
Tin piute	*Gilia aggregata*
Utah loco	*Astragalus utahensis* and other species
White fir	*Abies concolor*
Wild-cabbage	*Caulanthus crassicaulis*
Wild-daisy	*Erigeron caespitosus*
Winterfat (white sage)	*Eurotia lanata*

LITERATURE CITED

(1) HARRIS, W. R.
1909. THE CATHOLIC CHURCH IN UTAH, II. (Journals kept by Silvestre Velez de Escalante and Francisco Antanasio Dominguez.)

(2) HAYDEN, F. V.
1873. SIXTH ANNUAL REPORT OF THE UNITED STATES GEOLOGICAL SURVEY OF THE TERRITORIES, EMBRACING PORTIONS OF MONTANA, IDAHO, WYOMING, AND UTAH; BEING A REPORT OF PROGRESS OF THE EXPLORATIONS FOR THE YEAR 1872. 844 pp., illus.

(3) HITCHCOCK, A. S.
1935. MANUAL OF THE GRASSES OF THE UNITED STATES. U. S. Dept. Agr. Misc. Pub. 200, 1040 pp., illus.

(4) KEARNEY, T. H., BRIGGS, L. J., SHANTZ, H. L., MCLANE, J. W., and PIEMEISEL, R. L.
1914. INDICATOR SIGNIFICANCE OF VEGETATION IN TOOELE VALLEY, UTAH. Jour. Agr. Res. 1: 365–417, illus.

(5) SIMPSON, J. H.
1876. REPORT OF EXPLORATIONS ACROSS THE GREAT BASIN OF THE TERRITORY OF UTAH . . . IN 1859. Engin. Dept., U. S. Army. 518 pp., illus.

(6) STANSBURY, HOWARD.
1853. EXPLORATION AND SURVEY OF THE VALLEY OF GREAT SALT LAKE OF UTAH, INCLUDING A RECONNOISSANCE OF A NEW ROUTE THROUGH THE ROCKY MOUNTAINS. 495 pp., illus. (U. S. Cong. 32d, Spec. sess. March 1851, S. Exec. Doc. 3.)

(7) STEWART, GEORGE, and HUTCHINGS, S. S.
1936. THE POINT-OBSERVATION-PLOT (SQUARE-FOOT DENSITY) METHOD OF VEGETATION SURVEY. Amer. Soc. Agron. Jour. 28: 714–722, illus.

(8) ——— and KELLER, WESLEY.
1936. A CORRELATION METHOD FOR ECOLOGY AS EXEMPLIFIED BY STUDIES OF NATIVE DESERT VEGETATION. Ecology 17: 500–514.

TOXICITY TO SHEEP OF LEAD ARSENATE AND LEAD ARSENATE SPRAY RESIDUES[1]

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INTRODUCTION

Occasionally reports are received at the Washington station of the illness or death of livestock, principally cattle and sheep, that have been pastured in sprayed orchards during the fall. Many of these losses have occurred where veterinary service was not available and have been attributed to anthrax or to some other acute infectious disease. These diagnoses, however, have not been confirmed by members of the veterinary profession or by laboratory findings.

During the last 2 years the authors have investigated two such outbreaks, one of which resulted in the loss of approximately 1,000 sheep and the other in the loss of 23 feeder steers (8a).[3] The post-mortem pathology and the essentially negative nature of the bacteriological findings, together with the case histories, pointed to acute chemical poisoning rather than to an acute infectious disease.

The orchard grasses from the area on which the sheep were known to have fed showed on analysis 0.44 percent of arsenic (As) and 1.44 percent of lead, which is more than 4,000 times the established tolerance for foods. The leaves of the trees on which the steers are known to have fed contained 0.0045 percent of arsenic.

Analyses of the rumen contents of the last of the poisoned animals to die and of moribund survivors revealed the presence of sufficient lead and arsenic to account for 30 gm. of lead arsenate in the sheep and 38 gm. in the steers. Since sufficient lead arsenate had been absorbed to cause severe illness or death and some had been eliminated from the body, it is evident that considerably larger amounts had been ingested.

Approximately 15 percent of the poisoned sheep and 80 percent of the poisoned steers made at least a temporary recovery from the effects of the lead arsenate. If it be assumed that some of the animals which were made ill, but which did not succumb within the following 3 months, consumed almost as much as did the last of the animals to die of acute poisoning, the amounts of lead arsenate consumed by these survivors were considerably greater than the reported minimum lethal doses of lead arsenate for sheep and cattle.

REVIEW OF THE LITERATURE

The literature contains numerous references to poisoning with lead and with arsenic. For bibliographies the reader is referred to Osborn

[1] Received for publication August 7, 1939. Published as Scientific Paper No. 414, College of Agriculture and Experiment Station, State College of Washington.
[2] The writers express their appreciation to D. Finch and L. Murphy for assistance in caring for the experimental animals, and to F. Van Amburgh for the analytical work.
[3] Italic numbers in parentheses refer to Literature Cited, p. 328.

and Pease,[4] Pease and Osborn,[5] and Aub (*2*). Most of the published work, however, is based upon the toxicity of lead and arsenic fumes or dust; the toxicity of lead and arsenic compounds other than lead arsenate; the effects of these compounds upon rats and other laboratory animals and upon dosages far below the levels known to have been consumed by the sheep and cattle that died after having grazed upon the forage in heavily sprayed orchards. In most of the experiments in which large animals were used, the experimental animals were allowed to graze areas of lead arsenate-sprayed forage. From such experiments, the exact amounts of lead arsenate eaten by the animals cannot be ascertained.

Data obtained from inhalation of lead and arsenic dust or fumes probably are not applicable to the oral administration of these substances.

Perhaps the lower incidence of plumbism in animals consuming lead-contaminated forage than in people exposed to lead in the industries may be explained by the difference in the ways in which exposure occurs. Lanza (*8*, *p. 85*) pointed out that—

> most of the industrial exposure arises from dust and fumes that are breathed into the lungs and upper respiratory tract, where absorption and excretion involve entry into the systemic circulation. Lead that is ingested may be excreted unchanged and, even if absorbed, may be carried to the liver and excreted in the bile.

Cardiff (*3*) believes that the poisoning of horses, when hay from heavily sprayed orchards is fed, results from the inhalation of the dried spray residues rather than from the consumption of the lead arsenate.

Thomas and Shealy (*15*) found that while lead arsenate will reproduce death in chickens when fed in large quantities, a chicken may consume as much as 13 grains per day for 60 days without suffering any ill effects.

Coulson, Remington, and Lynch (*4*) found that rats fed shrimp which naturally contained 17.70 parts per million of arsenic stored only 0.13 mg. of arsenic in 3 months, while in a parallel experiment rats which received a similar amount of As_2O_3 in their diet stored 3.73 mg. After as long as 12 months on these diets, neither group showed any evidence of poisoning.

Although Talbert and Tayloe (*14*) do not feel that their data are sufficient to permit conclusions regarding lead poisoning from the consumption of sprayed apples, they do state that there is little likelihood of a person consuming as spray residue on apples enough arsenic at one time or over an extended period to be injurious.

Husband and Duguid (*7*) found that cattle survived 20 grains of arsenic fed as sodium arsenite. Thirty grains killed in some cases and larger amounts were fatal. Cattle died after consuming pasture grass sprayed with 1.5 pounds of sodium arsenite per acre. Arsenic was found in the intestinal contents and in several organs. Husband and Duguid state that analyses of the omasum and kidney give more reliable information than those of other organs in arsenic poisoning.

One of the earliest experiments in which exact amounts of lead arsenate were fed was conducted by Paige (*10*), who used a lead arsen-

[4] OSBORN, R. A., and PEASE, V. A. SELECTED REFERENCES ON THE TOXICITY OF ARSENIC, 1901–1934. U. S. Bur. Chem. and Soils, Food Res. Div. 45 pp. 1934. [Mimeographed.]

[5] PEASE, V. A., and OSBORN, R. A. SELECTED BIBLIOGRAPHY ON METALLIC CONTAMINATION OF FOODS, WITH SPECIAL REFERENCE TO CONTAINERS AND COOKING UTENSILS. U. S. Bur. Chem. and Soils, Food Res. Div. 37 pp. 1936. [Mimeographed.]

ate paste somewhat different in composition from that now applied to orchards. Since the lead arsenate paste contained about 9 percent of arsenic and 32.5 percent of lead, the quantities of the paste that he administered should be reduced to approximately one-half his stated value to be comparable to modern dry lead arsenate.

All the cattle that Paige used were tuberculous, which may have decreased their ability to withstand toxic doses and to recover after they were poisoned. In his experiments Paige fed a 600-pound cow 1 gm. of lead arsenate paste per day for 26 days and observed no symptoms of lead poisoning. Following a 4-day interval she was again given 1 gm. per day and died after 10 days. A 1,015-pound cow given 184 gm. of lead arsenate paste in daily doses of from1 ½ to 3 gm. over a period of 117 days, made an apparent recovery, but a single dose of 50.4 gm. 43 days later resulted in violent purgation and inappetence. She was autopsied 19 days later. Another cow weighing 600 pounds survived a single dose of 28.35 gm. of lead arsenate paste, and 96 days later was started on another series of tests in which 105 gm. were fed in 57 days. Two days later she was given a single dose of 19.8 gm. and slaughtered 9 days thereafter. Paige reports (*10, p. 190*):

> Principal lesions: small tubercular nodule in lobe of lung; indications of the irritant and poisoning action of arsenate of lead few. Blackening of the gums about the incisor teeth quite pronounced in this animal; not found in any of the others.

Green and Dijkman (*6*) found that in most cases the oral administration of 0.2 to 0.3 gm. of arsenic trioxide, supplied as sodium arsenite, produced no symptoms of poisoning in sheep and that death was uncertain when less than 0.5 gm. was given.

Seddon and Ramsay (*12*) studied the toxicity of various compounds of lead and of arsenic for sheep by mixing the drug with small quantities of bran and sirup and administering it as an electuary. For 80- to 100-pound sheep they found fatal doses to be as shown in table 1. Of especial interest are the differences in the amounts of arsenic contained in the toxic and the nontoxic doses when the arsenic was given in various chemical combinations. The data indicate the more soluble compounds of arsenic to be the more toxic.

TABLE 1.—*Lethal doses of various arsenic and lead compounds when administered to sheep in bran and sirup as an electuary*

[From work of Seddon and Ramsay (*12*)]

Dose and compound administered	Size of dose		Poison contained in dose		
			As_2O_3	As	Pb
	Grains	*Grams*	*Grams*	*Grams*	*Grams*
Fatal doses:					
Sodium arsenite	13.3	0.862	0.577	0.437	.....
Paris green	20.0	1.296	.715	.542	.....
Arsenic acid	13.3	.862	.551	.417	.....
Lead arsenate	60.0	3.888	1.251	.947	2.317
Lead oxide	180.0	11.664	.....	.....	9.691
Lead acetate	270.0	17.496	.....	.....	9.321
Nontoxic doses:					
Sodium arsenite	6.6	.428	.287	.217	.....
Arsenic acid	6.6	.428	.274	.208	.....
Lead arsenate	40.0	2.592	.835	.632	1.545
Lead acetate	216.0	13.997	.....	.....	7.457
Lead sulfate	60.0	3.888	.....	.....	2.655

O'Kane, Hadley, and Osgood (*9*) fed lead arsenate to sheep and other animals. The lead arsenate was sprayed on green foliage which the sheep consumed directly. The exact amount consumed was, therefore, not known. With two sheep that were estimated to have received a little over 3 gm. of lead arsenate each per day for 45 days, any symptoms of poisoning disappeared by the end of 30 days. Sheep receiving 7 gm. per day showed definite symptoms of poisoning, and by the fifth day the feeding was discontinued. These animals recovered in about 3 days. Another lot received about 12 gm. (estimated) per day of lead arsenate. These animals showed prompt and definite symptoms of poisoning. One died on the fourth day. The lead arsenate feeding was discontinued with the other sheep, which appeared quite normal after 4 days and finally recovered.

Reeves (*11*) reports that sheep were poisoned by 4 to 8 grains of arsenic when it was fed daily.

EXPERIMENTAL PROCEDURE

A series of experiments was outlined in which sheep were fed lead arsenate at various levels daily. The sheep used were healthy, vigorous lambs weighing approximately 80 pounds (36.29 kg.). Each animal was maintained in an individual metabolism crate at the College of Veterinary Medicine throughout the experiment. The basal ration fed to all experimental animals consisted of rolled oats 3 parts, rolled barley 4 parts, and alfalfa hay 14 parts, with salt ad libitum. Since fallen apples are usually consumed by animals pastured in orchards, a measured quantity of Jonathan apples was included in the ration of some of these animals. The animals were accustomed to the metabolism crates and to the basal ration and were making daily gains before the administration of arsenic was started.

During the experimental period the urine and feces were collected daily, sampled, placed in 2-quart jars to which thymol was added, and the samples placed in cold storage. The daily feed and water consumption were accurately measured. The daily dose of lead arsenate for each animal was divided into two portions which were placed in gelatin capsules and given morning and night, so that the exact amount of lead arsenate taken into the body was definitely known. The lead arsenate used was a well-known brand which is widely used in orchard spraying in Washington State. It contains 59.6 percent of lead and 21.5 percent of arsenic.

For the determination of lead the tentative methods of the Association of Official Agricultural Chemists (*1*) were followed except that in preparing the samples for analysis nitric and perchloric acids were used according to methods developed in the laboratory of the Division of Chemistry by Gerritz (*5*). The lead was isolated by direct diphenylthiocarbazone extraction or, when necessary, the lead was precipitated and isolated as the sulfide, dissolved in 1 percent nitric acid and extracted with diphenylthiocarbazone. The quantitative readings were then made by colorimetric comparison according to the method developed for spray-residue determination. Arsenic was determined on an aliquot of the nitric and perchloric acid-digested sample either by the bromate or the Gutzeit method, depending upon the quantity of arsenic present.

EXPERIMENT 1

In the first experiment three sheep were fed 2 g. of lead arsenate daily. Two of these received 2 pounds of apples each day in addition to the basal ration and the third received only the basal ration. One of the sheep that received apples and the sheep that received only the basal ration died on the fourth day after each had consumed a total of 7 g. of lead arsenate. The remaining sheep, which had received apples, died after 1 week, having consumed a total of 15 g. of lead arsenate.

On autopsy the sheep that died on the fourth day were found to have hemorrhagic erosions of the rumen wall, which extended into the recticulum. Severe enteritis was present, especially in the duodenum. The lungs were pneumonic. In the third sheep the lesions were similar to those in the other two except that the kidneys had a metallic sheen.

As soon as the lead arsenate was given to the sheep, their feed consumption decreased by approximately 80 percent, and the amount of water consumed was also markedly reduced.

The daily excretion of lead and arsenic, expressed in milligrams, is shown in table 2. While there are variations in the quantity of lead and arsenic eliminated from day to day and in the quantity eliminated by different sheep, these data do not show any correlation between the inclusion of apples in the diet and the rate of lead or arsenic elimination. The data are made more difficult to interpret by the marked reduction in feed and water intake and a somewhat parallel reduction in the amount of feces and urine following the administration of the lead arsenate.

Much of the arsenic eliminated was through the urine, although the feces contained appreciable amounts. From 44 to 48 percent remained in the digestive tract at the time of death, while from 54 to 60 percent of the total amount of arsenic fed to these sheep was accounted for by analysis of the contents of the digestive tract, the liver, kidneys, and the wool.

More lead was eliminated from the body through the feces than through the urine. From 60 to 77 percent remained in the digestive tract, while from 62 to 79 percent of the lead consumed was accounted for by analysis of the digestive tract, the liver, and the kidneys.

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TABLE 2.—*Total lead arsenate ingested by sheep fed 2 gm. daily; quantity of arsenic and lead excreted daily; and total quantity found at autopsy*

Sheep	Total lead arsenate ingested [1]		Arsenic and lead excreted—														Total found at autopsy in—					
			Mar. 16		Mar. 17		Mar. 18		Mar. 19		Mar. 20		Mar. 21		Mar. 22		Liver		Kidney		Stomach	
	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb	As	Pb
Urine:	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
2 [2]	3.24	8.95	34.2	2.4	42.0	1.6	42.6	1.4	58.8	3.0	18.3	1.1	45.7	3.0	39.8	16.0	7.3	2.9	1.7	1.2	1,560.0	5,958.0
3	1.51	4.18	35.1	2.9	18.1	1.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	27.3	7	1.7	1.4	716.0	3,230.0
4 [2]	1.51	4.18	25.8	4.1	31.7	4.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7.8	6	1.3	1.1	666.0	2,512.0
Feces:																						
2 [2]			9.2	54.9	6.0	26.6	27.3	114	25.2	80.6	.....	.....	18.4	97.2								
3			2.0	36.5	7.2	32.3	.....	.....	.....	.....	.....	.....	.....	.....								
4 [2]			1.2	30.9	3.4	54.0	.....	.....	.....	.....	.....	.....	.....	.....								

[1] Lead arsenate first fed Mar. 15, 1938.
[2] Apples included in ration.

EXPERIMENT 2

Since the daily administration of 2 gm. of lead arsenate had proved lethal to three sheep in from 2 to 7 days, a second experiment was conducted in which the amounts of lead arsenate given daily were 1, 0.5, and 0.25 gm. Two sheep, one of which received the basal ration only and the other received apples in addition, were placed on each level. A seventh sheep was included as a control.

One of the sheep that received 1 gm. of lead arsenate daily and apples as as part of the ration died in 6 days; the other sheep that received 1 gm. of lead arsenate daily and only the basal ration died in 7 days. The lethal amount of lead arsenate fed at the rate of 1 gm. per day was, therefore, 6 or 7 gm. These figures represent 1.29 and 1.51 gm. of arsenic (As) (equivalent to 1.70 and 1.99 gm. if expressed as As_2O_3). It should be noted that this is approximately the same total quantity that killed two of the three sheep in experiment 1 where 2 gm. of lead arsenate were fed daily. The 1.5 gm. of arsenic (As) for an 80-pound sheep is equivalent to 41 mgm. per kilogram, or 192 mgm. of lead arsenate per kilogram.

One of the sheep receiving 0.5 gm. of lead arsenate per day died after 35 days, having consumed a total of 17.5 gm. of lead arsenate, equivalent to 3.7 gm. of arsenic (As). The other sheep receiving 0.5 gm. of lead arsenate per day died after 79 days, having consumed 39.5 gm. of lead arsenate, equivalent to 8.4 gm. of arsenic.

One of the sheep receiving 0.25 gm. of lead arsenate daily died after 35 days, having received 8.75 gm. of lead arsenate containing 1.9 gm. of arsenic. The other sheep survived 94 days on this level, when the lead arsenate feeding was discontinued, and 10 months later when slaughtered was in good condition and showed no evidence of injury. This sheep consumed 23.5 gm. of lead arsenate during the 94 days representing 5 gm. of arsenic. The excretion of lead and arsenic by the sheep in experiment 2 is shown in table 3.

TABLE 3.—*Daily excretion of lead and arsenic by sheep that received ¼, ½, and 1 gm. of lead arsenate daily*

Sheep	Quantity of lead arsenate fed [2]	Arsenic and lead [1] excreted—																					
		Apr. 25		Apr. 27	Apr. 29		May 1	May 3		May 5	May 7		May 9	May 11		May 13		May 15		May 17	May 19		May 21
		As	Pb	As	As	Pb	As	As	Pb	As	As	Pb	As	As	Pb	As	Pb	As	Pb	As	As	Pb	As
Urine:	*Gram*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1	¼	2.82	------	4.87	------	------	6.25	1.83	------	0.39	0.72	------	1.0	1.02	------	1.35	------	1.18	------	0.34	0.79	------	0.36
2	½	3.6	4.0	------	------	------	5.6	6.6	------	11.6	1.89	------	1.94	1.37	------	.74	------	5.6	------	.98	.86	------	.30
3 [3]	¼	1.92	------	7.51	0.33	------	.79	.54	------	.70	.75	------	.14	1.82	------	.19	------	.28	------	.68	.10	------	2.87
4 [3]	½	1.03	------	2.10	1.39	------	------	1.01	------	1.12	.52	------	.79	.84	------	1.23	------	.33	------	.92	.25	------	1.52
5 [3 4]	1	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
6 [5]	1	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
Feces:																							
1	¼	6.73	89.0	------	6.10	97.0	------	2.42	46.0	------	5.3	104.0	------	6.8	28.0	------	------	5.0	27.0	------	30.2	168.0	------
2	½	9.86	149.0	------	13.7	242.0	------	16.1	142.0	------	23.3	370.0	------	17.0	189.0	------	88.0	------	------	------	20.9	76.0	------
3 [1]	¼	6.30	104.0	------	9.08	120.0	------	15.7	195.0	------	3.79	32.0	------	5.6	28.0	------	------	12.3	57.0	------	20.3	63.0	------
4 [1]	½	1.56	132.0	------	15.0	220.0	------	15.9	258.0	------	17.6	265.0	------	18.9	240.0	------	------	16.3	286.0	------	17.6	280.0	------

[1] No lead determination made where data are lacking.
[2] Lead arsenate feeding began Apr. 24, 1938.
[3] Apples included in the ration.
[4] Died Apr. 29.
[5] Died Apr. 30.

As in experiment 1, the amount of arsenic eliminated daily in the urine varied widely, and there appeared to be no correlation between the amount fed per day and the amount eliminated. Essentially the same is true with regard to the elimination of arsenic and lead in the feces, although in general more arsenic was eliminated per day in the feces than in the urine. In the feces the ratio of the amount of lead eliminated to the amount of arsenic eliminated was much above the ratio of these two elements in lead arsenate. The actual weight of arsenic eliminated per day in the feces was greater than in the urine in experiment 2; the reverse was true in experiment 1.

Table 4 summarizes the total amount of lead and arsenic eliminated and the total remaining in the stomach and other organs.

The data show that only a small proportion of the ingested lead arsenate was eliminated. Of the four sheep in experiment 2 that lived for 35 days or more, one eliminated 16 percent of the arsenic intake, while two eliminated 4 and 5 percent. The elimination of lead varied from 46 to 12 percent.

Thirty-three to forty-two percent of the arsenic accounted for was found in the stomach contents, while 17 to 24 percent of the lead was found there. The total amount of arsenic accounted for ranged from 10 to 28 percent of the amount ingested while the total amount of lead that could be traced ranged from 32 to 61 percent. Analyses were made of the liver, kidneys, heart (table 4), and gall bladder, and in one case of the lungs, ribs, and other bones, bone marrow, and wool (table 5). The concentration in all the parts examined was small as compared with that in the stomach contents, which accounted for 4 to 38 percent of the arsenic ingested and 13 to 49 percent of the lead. The amounts found in individual sheep varied widely.

TABLE 4.—*Total amount of lead and arsenic eliminated by sheep and total found at autopsy in stomach and other organs*

Sheep No.	Total lead arsenate fed	Total ingested		Total eliminated in—				Total recovered in—							
		As	Pb	Urine, As	Feces		Total, As	Stomach contents		Liver		Kidney		Heart	
					As	Pb		As	Pb	As	Pb	As	Pb	As	Pb
	Grams	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1	23.0	5.0	13.7	49.28	140.1	1,695.2	189.4	263	1,500.0	4.5	9.4	1.8	3.5	0.11	0.30
2	17.0	3.7	10.1	97.16	437.1	4,664.9	534.2	226	680.0	5.5	8.8			.22	.40
3 [1]	9.0	1.9	5.4	37.24	271.44	2,225.6	308.7	332	1,280.0	.73	3.1	.14	2.1	.16	.42
4 [1]	39.0	8.4	23.3	28.0	434.20	6,242.6	462.2	265	1,200.0	5.6	5.3	.75	.44		
5 [1]	6.0	1.29	3.57					576	2,040.0	10.6	10.8	2.5	2.9		
6	7.0	1.51	4.17												

[1] Apples included in ration.

TABLE 5.—*Quantities of arsenic and lead found in lungs, bones, marrow, gall bladder, and wool of a sheep that was fed one-half of a gram of lead arsenate daily for 79 days*

[Results expressed as milligrams per 100 gm.]

Element	Quantity in—					
	Lungs	Ribs	Femur	Marrow from femur	Gall bladder	Wool
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
As	0.38	1.42	0.35	1.11	0.01	0.28
Pb	3.0	2.5	.23	1.6	.15	.74

The findings at autopsy were similar to those described for the sheep in experiment 1. In those sheep that lived long enough for the phenomenon to be observed it was found that the erythrocyte count rose at first but fell to low levels a few days before death. In the sheep that lived 35 days, the count rose from approximately 8,000,000 when the feeding of lead arsenate began, to 13,000,000 on the tenth day thereafter, and then dropped to 4,200,000 on the twenty-seventh day. The leucocyte count fluctuated within normal limits until bacterial infection set in, when in most cases it rose sharply. A lead line was observed on the fourteenth day. On the twenty-sixth day a foul diarrhea appeared and the day before death hemoglobinuria was observed. The animal died in a cachexic state after 35 days.

On post mortem no erosions were found on the mouth, esophagus, or other portions of the digestive tract. A distinct lead line was observed. Enteritis was present to only a moderate extent. Fatty degeneration of the heart was evident and the lungs were congested and pneumonic. The spleen was ruptured and swollen, with the pulp distinctly darkened. The kidneys were blue black in color and had a metallic sheen. They were so soft that removal without breaking was difficult. The marrow of the flat bones was black. Upon exposure to the air the marrow became dark red. In the meat-packing industry animals showing this condition are known as "black cutters."

On post-mortem examination, lesions similar to those in sheep fed lethal amounts of lead arsenate were found in the sheep that received 0.25 g. of lead arsenate and died at the end of 35 days. The second sheep fed this amount continued to receive it for 94 days, when the lead arsenate feeding was discontinued. This sheep was in excellent condition when slaughtered 10 months later and showed no evidence of the earlier consumption of 23.5 g. of lead arsenate. It is of particular interest to note that when this animal was killed the bones showed no evidence of darkened marrow.

DISCUSSION AND SUMMARY

The toxicity of arsenic and lead appear to be affected by the nature and the solubility of the compounds in which they occur and the means by which they enter the body. Sheep were killed by the oral administration of 0.4 to 0.5 g. of arsenic (As) in the form of arsenic trioxide (As_2O_3), but substantially larger amounts of arsenic (As) in the form of lead arsenate were required to kill. When lead arsenate was sprayed on foliage and allowed to remain for several weeks, still larger amounts were necessary to cause death. A materially larger quantity of lead

arsenate was required to kill when the sheep consumed it on sprayed foliage than when it was administered experimentally in capsules. This should be further checked by carefully controlled experimental work involving the feeding of foliage carrying spray residue applied some weeks before the beginning of the experiments. If the experiments should show a change in the form of the lead and arsenic on foliage, together with a change in toxicity, this might explain the survival of animals that consumed large amounts of lead arsenate on sprayed foliage.

Nine sheep were fed lead arsenate varying in amount from 2 gm. per sheep per day to 0.25 gm. Apples were included in some of the rations. The animals receiving 1 and 2 gm. per day of lead arsenate died within a few days, in most cases after having received about 1.5 gm. of arsenic (2 gm. As_2O_3). One animal that received 0.25 gm. of lead arsenate per day survived for 35 days, and another that received the same amount for 94 days continued to live and was in good condition at the end of 10 months, when it was slaughtered. A total of 1.5 gm. of arsenic in the form of lead arsenate appears to be approximately a lethal dose when fed in small amounts daily. This is equivalent to about 41.0 mgm. of arsenic (As) or 192 mgm. of lead arsenate per kilogram of body weight.

Analytical results on the stomach contents and on a number of organs, as well as on the urine and feces, did not account for all the lead and arsenic consumed. The proportion of the arsenic consumed which was eliminated was rather small; the proportion of the lead eliminated was somewhat larger. The results obtained suggest a relation between the quantity consumed per day and the proportion eliminated. When larger quantities were consumed a larger proportion was eliminated in the urine; when smaller quantities were consumed a larger proportion was eliminated in the feces. Even in the latter case the quantity of lead was greater than that of arsenic. Paradoxically, the sheep that eliminated the smallest percentage of both arsenic and lead continued to live for 3 months. A larger proportion of lead than of arsenic was found in the stomach contents, presumably because of the difference in solubility. A larger proportion of the lead and arsenic remaining in the body was found in the stomach contents than elsewhere, although more was eliminated than remained in the stomach. The inclusion of apples in the ration had no apparent effect on the toxicity or elimination of lead and arsenic. Small quantities of these elements were found in the liver, kidney, heart, lungs, gall bladder, bones and marrow, and wool.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1935. official and tentative methods of analysis . . . Ed. 4, 710 pp., illus. Washington, D. C.

(2) Aub, Joseph C., Fairhall, Lawrence T., Minot, A. S., and Reznikoff, Paul.
1926. lead poisoning. With a chapter on the prevalency of industrial lead poisoning in the United States, by Alice Hamilton . . . 265 pp., illus. Baltimore.

(3) Cardiff, Ira D.
1937. observations with reference to arsenic on apples and other foodstuffs. Wash. State Hort. Assoc. Proc. 33: 153–168.

(4) Coulson, E. J., Remington, Roe E., and Lynch, Kenneth M.
1934. toxicity of naturally occurring arsenic in foods. Science 80: 230–231.
(5) Gerritz, H. W.
1935. digesting biological materials for calcium and phosphorus analysis. Indus. and Engin. Chem., Analyt. Ed. 7: 167–168.
(6) Green, Henry Hamilton, and Dijkman, Cornelis Derksen.
1919. some experiments on fate of arsenic in the animal body. So. African Jour. Sci. 15 (So. African Assoc. Adv. Sci. 1918): [640]–651.
(7) Husband, A. D., and Duguid, J. F.
1934. the toxicity to grazing animals of grass sprayed with a solution of sodium arsenite. Rhodesia Agr. Jour. 31: 25–39, illus. [Abstract in Expt. Sta. Rec. 71: 241.]
(8) Lanza, A. J.
1935. epidemiology of lead poisoning. Amer. Med. Assoc Jour. 104: [85]–87.
(8a) McCulloch, Ernest C.
1940 lead-arsenate poisoning of sheep and cattle. Amer. Med. Vet. Assoc. Jour. 96 (756): 321–326 illus.
(9) O'Kane, W. C., Hadley, C. H., Jr., and Osgood, W. A.
1917. arsenical residues after spraying. N. H. Agr. Expt. Sta. Bul. 183, 62 pp., illus.
(10) Paige, Jas. B.
1909. cattle poisoning from arsenate of lead. Mass. Agr. Expt. Sta. Ann. Rpt. (1908) 21 (pt. 2): 183–199.
(11) Reeves, Geo. I.
1925. the arsenical poisoning of livestock. Jour. Econ. Ent. 18: 83–90.
(12) Seddon, J. R., and Ramsay, A. A.
1933. toxicity of certain arsenic and lead compounds for sheep. N. S. Wales Dept. Agr. Vet. Res. Rpt. 6 (pt. 3, December 1931): 113–118.
(13) Society of Public Analysts.
1934. bibliography of the more important heavy metals occurring in food and biological materials (for the years 1921 to 1933 inclusive). 30 pp. London.
(14) Talbert, T. J., and Taylor, W. L.
1933. some results from feeding spray chemicals to albino rats. Mo. Agr. Expt. Sta. Res. Bul. 183, 19 pp., illus.
(15) Thomas, E. F., and Shealy, A. L.
1932. lead arsenate poisoning in chickens. Jour. Agr. Res. 45: 315–319.

COMPARISON OF A CHEMICAL AND A BIOCHEMICAL METHOD FOR DETERMINING THE BIOLOGICAL VALUE OF PROTEINS AND AN EVALUATION OF THE ENDOGENOUS NITROGEN [1]

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INTRODUCTION

Since the discoveries that animals require specific amino acids and that amino acid distribution in proteins differs with a resultant difference in nutritive quality, a chemical method for determining the nutritive value of proteins has been sought. Almquist, Stokstad, and Halbrook (*1*)[2] proposed a chemical method which differed from any previously used. Analyses of animal protein concentrates were made for intact protein, protein decomposition products, indigestible protein, and hot-water-soluble protein. A formula was then used to give a numerical "protein quality index" (*1, p. 205*).

The purpose of the present study was to compare the method of Almquist et al. with the Mitchell and Carman (*10*) modification of the Thomas-Mitchell (*8*) nitrogen-retention method of determining the biological value of protein. The digestion of the protein-containing materials was compared in vitro and in vivo. In addition, a comparison was made between the endogenous urinary nitrogen output when corrected according to body weight and when corrected according to body surface area (*12*).

PROTEIN-CONTAINING MATERIALS

The protein-containing materials studied are given in table 1. The cereals and cereal protein concentrates were commercial products and were used without purification. Soybean meal 1 was a solvent-extracted meal at 60° C. that had been heated after extraction to 110° for 30 minutes to drive off the residual solvent. Soybean meal 2 was a portion of meal 1 that had been heated at 110° for 15 hours after extraction. It had the characteristic straw color of heated soybean meals. The dry whole-egg and liver meals were commercial preparations that had been soaked in alcohol, extracted with ether for 48 hours and air-dried. The tankage, a commercial meat and bone product having about 50 percent of crude protein, was soaked in 95-percent ethanol, extracted with ethyl ether for 48 hours, and air-dried. The casein was a commercial casein which was purified twice by dissolving in ammonia, running through a supercentrifuge, and reprecipitating with a mixture of acetic and hydrochloric acids; it was then extracted with alcohol for 24 hours, with ether for 48 hours,

[1] Received for publication October 4, 1939. Paper No. 1681, Scientific Journal Series, Minnesota Agricultural Experiment Station. The data in this paper are taken from a thesis presented by Floyd C. Olson in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, 1938. The original data are on file in the Division of Biochemistry, University of Minnesota.

[2] Italic numbers in parentheses refer to Literature Cited, p. 341.

and dried at 40° in vacuum. The crude-protein content of the prepared foods is given in table 1.

TABLE 1.—*True digestibility and biological value of proteins tested by the nitrogen-retention method*

Source of protein	Crude protein content as prepared	Proportion in diet	Tests	True digestibility		Biological value	
				Average with standard error	Standard deviation	Average with standard error	Standard deviation
	Percent	*Percent*	*Number*	*Percent*		*Percent*	
Dried whole egg	77.18	8	12	97.9±0.17	0.58	93.9±0.82	2.84
Casein	91.68	8	11	99.7± .35	1.16	62.9±1.83	6.09
Whole wheat	13.40	8	11	91.7± .59	1.97	47.2±1.28	4.24
Wheat gluten	76.38	8	10	99.3± .21	.66	41.9±1.44	4.56
Whole corn	10.06	8	12	91.3± .53	1.83	52.5±1.10	3.80
Corn-gluten meal	42.63	8	10	96.7± .41	1.30	41.8±1.71	5.40
Liver meal	76.25	8	12	88.3± .40	1.38	56.5± .84	2.90
Tankage	56.19	12	14	83.3± .50	1.88	38.1±1.63	6.10
Soybean meal 1	47.70	8	10	83.9±1.10	3.48	61.1±1.16	3.68
Soybean meal 2	46.00	8	10	84.3± .86	2.70	68.5±1.06	3.35

EXPERIMENTAL METHODS

NITROGEN BALANCES

Two different series of nitrogen-balance experiments were run. The first series was performed in the Division of Agricultural Biochemistry, University of Minnesota. The metabolism cages and collection of excreta were essentially as described by Mason and Palmer (*7*). To prevent loss of scattered feed a porcelain feed dish was set into the center of a 1-pound coffee can, the side of which was cut down in such a way that wire netting placed over the top gave an incline of about 40°.

The biological values of the tankage and soybean meals 1 and 2 were determined in the Department of Agricultural Chemistry at Ohio State University, where a different technique was necessary because of a different type of cage. The technique used here was essentially that of Mitchell (*8*) except that the filter paper containing the absorbed urine was submitted to direct nitrogen determination, blanks being run on the same amount of similar filter paper.

The experimental protein rations furnished 4.5 percent of dry whole-egg protein in the nitrogen-free periods, and 8 percent of protein in the protein-feeding periods, except for the tankage which was fed at a 12-percent level in order to promote growth. To this was added 4.5 percent of Hawk and Oser (*5*) salt mixture, 1 percent of NaCl, 2 percent of cod-liver oil, filtered butterfat to give a total fat content of 10 percent, agar to give a crude-fiber content of about 2 percent, 0.3 percent of vitamin B_1 concentrate,[3] and tapioca dextrin to complete the 100 percent.

In the first series young male rats only were used from the highly inbred strains in the rat colony of the Division of Biochemistry, University of Minnesota. In the second series the rats, including eight females, were purchased from a commercial breeder.

All the experiments involved 4-day transition periods and 7-day collection periods. Two experimental protein-containing rations were

[3] A commercial product, containing 105 International Units of vitamin B_1 per gram and 9.04 percent of nitrogen.

fed in the periods between initial and final nitrogen-free periods. The rats were so divided that one-half the products were tested in the first protein-feeding period and one-half in the second.

The appetite of the rats during the nitrogen-free periods was good, and they all gained slightly in weight. The food intake on the wheat gluten and corn-gluten meal was rather low, but all rats which did not gain weight were discarded.

CHEMICAL ANALYSES

The same protein products used in the feeding experiments were analyzed by the procedure of Almquist and associates (*1*). In this method pepsin HCl alone is the digesting agent. In order to test its completeness, peptic [4] digestion was followed by tryptic,[5] the solution being made alkaline to an alkalinity of 0.5-percent sodium carbonate with solid sodium carbonate, 25 ml. of 1.5-percent trypsin solution was added, and the sample shaken by machine for another 24 hours at 40°C. The insoluble material was analyzed for nitrogen. Blanks for the enzymatic digestion were run in the same manner as when protein was present. The activity of the enzyme preparations is indicated by the fact that they effected nearly 100-percent digestion of casein.

It was noticed in the peptic-tryptic digestion of liver meal and tankage that a copious reddish-brown precipitate formed on neutralizing with sodium carbonate. This was thought to be porphyrin, the hydrochlorides of which are soluble but which are insoluble in neutral or basic solution. A nitrogen analysis of the porphyrin precipitate was made in separate experiments to determine how much of this fraction classed as digestible by the procedure of Almquist et al. (*1*) would be classed as indigestible in the pepsin-trypsin digestion.

EXPERIMENTAL DATA

The endogenous urinary nitrogens in the nitrogen-balance experiments, corrected for body weight, are presented graphically in figure 1. The values used for plotting figures 1 and 2 were taken from table 2 and a third *N*-free period on 11 rats not shown in this table. The formula for the regression line was first calculated in terms of logarithms and then converted into arithmetical terms. The formula is $N=135.6\ W^{-0.469}$], where N equals endogenous urinary nitrogen in milligrams and W equals body weight in grams. The negative slope of the curve indicates that the endogenous urinary nitrogen per unit of body weight decreases with an increase in body weight. This formula differs considerably from Ashworth's (*3*) formula, $N=69.3\ W^{-0.274}$ calculated for certain of Mitchell's data on young rats.

Smuts (*12*) reported that the endogeneous urinary nitrogen is more closely related to body surface than to body weight. Figure 2 shows the same data as figure 1 plotted in relation to the body surface, this being calculated by Lee's (*6*) formula, $S=12.54\ W^{0.60}$ where S is the body area in square centimeters and W is body weight in grams. The formula for the regression line in figure 2 is $N=5.61\ A^{0.069}$, where N is the endogenous nitrogen in milligrams and A is body-surface area in square centimeters. When the data are calculated according to Fisher's (*4*) *t*-value, the exponent is shown to be not significantly different from zero.

[4] Powdered pepsin, U. S. P. X., Merck, was employed. [5] Trypsin, Difco, 1:110, was employed.

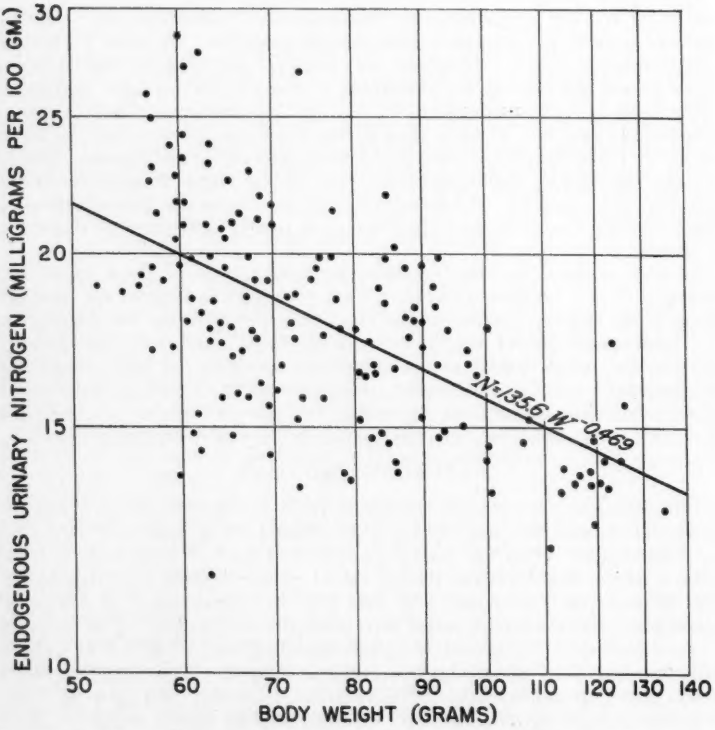

FIGURE 1.—Regression curve of milligrams of endogenous urinary nitrogen per 100 gm. of body weight plotted against body weight.

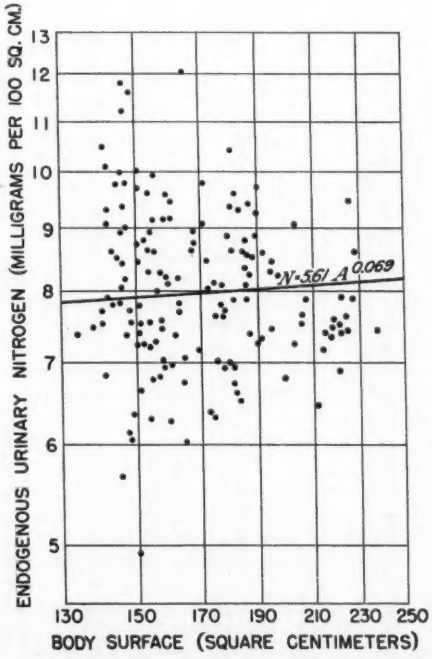

FIGURE 2.—Regression curve of milligrams of endogenous urinary nitrogen per 100 cm.^{2} of body surface plotted against body-surface area.

TABLE 2.—*Summary of endogenous urinary nitrogen data from which figures 1 and 2 were drawn*

Rat No.	First N-free period				Second N-free period			
	Average weight of rat	Food consumed daily	Endogenous urinary N daily	Endogenous urinary N per 100 cm.² of body surface	Average weight of rat	Food consumed daily	Endogenous urinary N daily	Endogenous urinary N per 100 cm.² of body surface
	Grams	*Grams*	*Milligrams*	*Milligrams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*
1	69	7.6	15.2	9.55	125	10.5	17.9	7.88
2	65	7.4	14.7	9.58	127	10.6	19.8	8.63
3	65	7.1	11.5	7.49	124	10.9	21.4	9.46
4	54.5	5.9	10.3	7.46	106.5	8.1	15.7	7.61
5	67	6.7	12.5	8.00	103.5	6.3	14.7	7.24
6	66.5	6.6	14.2	9.13	106	6.1	15.5	7.53
7	61.5	6.4	9.0	6.06	92.5	5.9	18.5	9.75
8	61	6.3	9.1	6.16	104.5	6.1	18.4	9.02
9	65.5	6.8	9.7	6.29	81	5.1	12.3	7.02
10	71	7.3	13.3	8.22	119.5	8.2	15.3	6.91
12	61.5	6.8	9.5	6.40	108	8.1	16.4	7.88
13	65	6.8	12.7	8.27	86	6.2	12.2	6.72
14	67.5	8.2	13.5	8.60	87.5	8.0	17.1	9.32
15	63	7.2	12.7	8.43	81	7.0	13.3	7.60
16	64.5	7.7	13.5	8.84	83	6.9	13.8	7.77
17	65.5	8.3	13.8	8.95	88.5	8.9	15.4	8.34
18	60.5	7.3	13.2	8.98	72.5	6.8	12.6	7.69
19	58	6.7	12.4	8.65	78.5	8.0	13.8	8.03
20	68	7.4	11.8	7.48	120.5	9.6	16.5	7.42
21	68.5	7.3	11.1	7.01	86	6.0	12.0	6.61
22	62.5	6.7	13.1	8.74	73.5	5.5	11.6	7.02
23	59	5.8	12.4	8.56	70	4.9	11.2	6.98
24	64	7.3	11.0	7.24	79	5.0	11.0	6.38
25	64	7.5	10.1	6.64	111	8.1	13.7	6.47
26	77.5	8.3	15.4	9.03	96.5	5.6	14.5	7.46
27	75	8.0	14.4	8.61	92.5	6.8	13.7	7.22
28	65.5	7.5	11.1	7.20	80	5.0	11.0	6.33
29	69.5	6.7	11.1	6.95	83	5.2	12.3	6.92
30	78.5	8.2	14.6	8.49	98.5	7.6	16.2	8.23
31	60.5	6.1	10.8	7.35	73	4.0	9.9	6.02
32	62	7.4	11.2	7.51	83	8.5	13.7	7.71
33	61.5	6.7	11.4	7.68	75.5	6.2	14.7	8.76
34	58.5	6.0	11.2	7.78	76.5	7.0	12.1	7.15
35	52	6.3	9.9	7.38	67	6.0	10.6	6.78
36	57.5	6.0	11.3	7.92	72	6.3	12.8	7.84
37	57.5	6.2	13.3	9.33	70	6.8	15.2	9.47
38	57.5	6.1	14.4	10.10	69.5	5.7	10.0	6.26
39	71	7.4	11.9	7.35	82.5	5.8	14.3	8.07
40	63	5.9	10.9	7.24	68.5	4.8	14.5	9.15
41	73	7.4	11.1	6.75	81.5	6.1	13.4	7.62
42	66.5	6.3	11.3	7.26	70	4.4	14.7	9.16
43	56.5	6.3	10.9	7.73	69.5	5.3	13.3	8.32
44	63	6.9	11.1	7.37	73.5	6.1	19.9	12.05
45	66	7.4	10.5	6.78	80	6.2	14.1	8.11
46	60	5.9	11.8	8.07	67.5	5.3	15.5	9.87
47	56	6.4	10.6	7.55	68	6.1	11.9	7.55
48	68	6.7	13.0	8.24	78	5.6	16.8	9.81
49	63.5	6.6	11.8	7.80	72	5.5	13.4	8.21
50	63	8.0	[1]	[1]	93	7.7	13.9	7.30
51	60	7.8	8.3	5.67	97	8.0	16.5	8.46
52	61	8.0	12.1	8.19	97	8.0	16.2	8.30
53	61	7.9	14.3	9.68	89	8.0	15.9	8.58
54	63	8.0	7.4	4.91	92	8.0	17.5	9.26
55	57	8.0	9.7	6.84	85	7.4	15.6	8.65
56	63	8.0	[1]	[1]	84	7.9	12.5	6.98
57	59	8.0	11.3	7.80	86	8.0	17.5	9.64
58	60	7.9	12.3	8.41	85	7.9	16.9	9.37
59	62	7.9	17.3	11.60	76	6.8	13.3	7.89
60	59	7.9	[1]	[1]	93	7.9	15.4	8.09
61	60	7.9	13.7	9.36	89	8.0	16.3	8.80
62	60	7.9	13.1	8.95	90	7.9	15.4	8.25
63	65	7.8	13.3	8.66	94	8.0	16.5	8.62
64	64	7.9	11.4	7.50	92	8.0	16.8	8.89
65	63	8.0	14.6	9.69	85	8.0	18.8	10.43
66	63	8.0	15.1	10.02	90	7.9	16.0	8.57
67	60	7.8	14.6	9.98	76	7.5	15.1	8.96
68	62	8.0	[1]	[1]	90	8.0	17.6	9.43
71	60	7.9	17.2	11.76	90	7.7	13.8	7.40
72	59	7.7	14.1	9.74	86	7.7	14.3	7.87
73	60.5	7.9	16.5	11.22	88	7.7	15.8	8.58
74	57	7.7	14.9	10.50	84	7.7	15.9	8.88
75	57	7.9	12.8	9.02	85	7.7	12.5	6.93

[1] First endogenous urinary N lost. Results based on second period.

When the data from Mitchell's laboratory, as used by Ashworth (*3*) are plotted in figure 3 on the body-surface-area basis, the regression formula $N=3.44\ A^{0.200}$ is obtained. This formula shows a larger increase in endogenous urinary nitrogen per unit of body surface with an increase in body surface than does the regression line in figure 2. An inspection of figure 2 shows a fair grouping of the values around the regression line. However, the values from Mitchell's laboratory show no grouping around the regression line but a wide scattering over the range of 5 to 17 mgm. per 100 cm.^{2} of body surface. This wide scattering of the points appears from the original data to be owing more to a variation between groups on different rations than to differences between individual animals. This would indicate that the variation was due to lack of complete control of the experimental procedure. For example, in some cases, even with whole egg in the ration, the animals lost weight, indicating that the food intake was not sufficient for the energy requirements. This would tend to increase the endogenous urinary nitrogen. Ashworth (*2*) reported that a 4-day preliminary period on a nitrogen-free ration is not sufficient for attainment of a low food intake. Mitchell and Beadles (*9*) reported a case in which the true endogenous level was not reached with a 4-day preliminary period.

However, it is to be noted that some of the values from Mitchell's data are for rats larger than those plotted in figure 2. Other determinations of endogenous urinary nitrogen on adult rats (not reported here) have given values considerably higher than 8 mgm. per 100 cm.^{2} of body surface even though a longer depletion period on a nitrogen-free ration was used. Smuts (*12*) found that rats weighing 150 gm. or more give an average endogenous urinary nitrogen of 15.40 mg. daily per 100 cm.^{2} of body surface. Calculations made from results obtained by Mason and Palmer (*7*) and by Lohn [6] from this laboratory on adult rats show values almost as high as those obtained by Smuts. Thus, it would seem that, whereas young rats of 50 to 100 gm. body weight show an endogenous urinary nitrogen output of 6 to 10 mg. per 100 cm.^{2} of body surface, older rats attain a considerably greater average output when calculated per unit body surface.

Table 1 gives the biological value and true digestibility with the standard errors and standard deviations for the test proteins. For the biological value calculations, the endogenous urinary nitrogen was corrected for changes in body surface area, and the metabolic fecal nitrogen was corrected for each gram of dry food intake.

Table 3 shows the significance of the differences between certain means, by using formulas given by Treloar [7] and referring to Sheppard's (*11*) tables for the relative deviate, x', in the determination of the probability for comparisons of biological value and digestibility. For comparisons of endogenous urinary nitrogen where correlation is expected, Fisher's (*4*) formula was used.

[6] LOHN, C. A STUDY OF THE EFFICIENCY OF FOOD METABOLISM FOR THE MAINTENANCE OF INBRED ANIMALS DIFFERING IN THEIR EFFICIENCY OF FOOD UTILIZATION DURING GROWTH. Thesis, Ph. D., Univ. Minn.

[7] TRELOAR, ALAN E. AN OUTLINE OF BIOMETRIC ANALYSIS. 3 v. in 1. Burgess Publishing Co., Minneapolis. 1935. [Mimeographed.] See pp. 28–29, and 56.

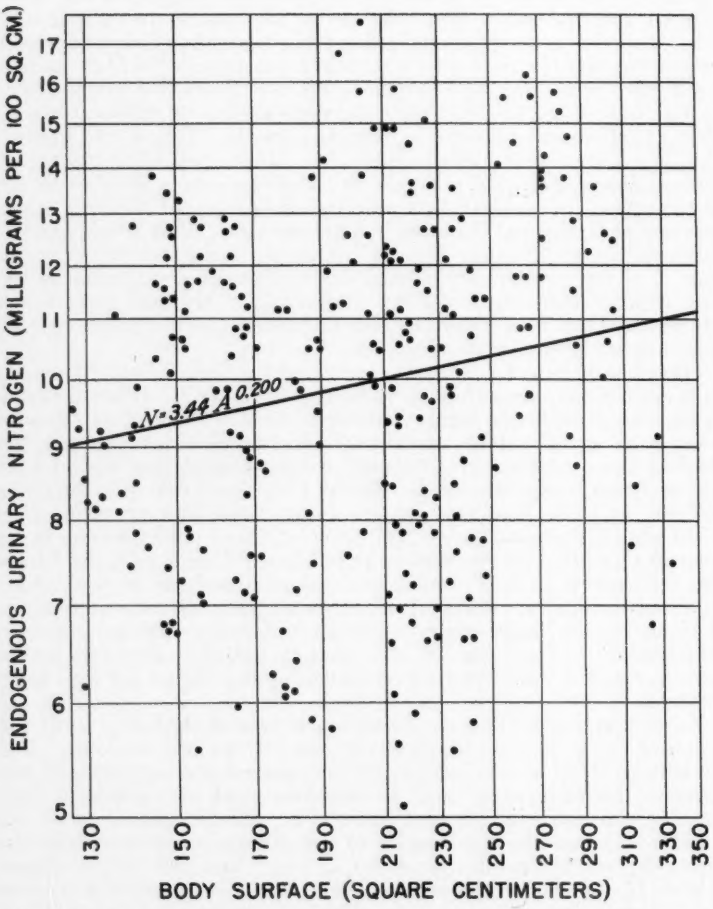

FIGURE 3.—Regression curve of milligrams of endogenous urinary nitrogen per 100 cm.^{2} of body surface plotted against body-surface area. Mitchell's data as cited by Ashworth (*3*).

TABLE 3.—*Determination of significance of differences in means for endogenous urinary-nitrogen and other data*

Item	Period or value	Mean	Standard deviation	Probability
		Milligrams		
Endogenous urinary nitrogen per 100 gm. of body weight.	First N-free period	19.6	3.4	0.001
	Second N-free period	17.1	2.6	
Endogenous urinary nitrogen per 100 cm.^{2} body surface.	First N-free period	8.2	1.4	.674
	Second N-free period	8.1	1.1	
Endogenous urinary nitrogen per 100 gm. of body weight, Mitchell's data (*3*).	First N-free period	22.1	5.0	.001
	Second N-free period	18.8	6.1	
Endogenous urinary nitrogen per 100 cm.^{2} of body surface, Mitchell's data (*3*).	First N-free period	10.2	2.2	.92
	Second N-free period	10.3	3.3	
		Percent		
Whole wheat	Biological value	47.2	4.2	.006
Wheat gluten	do	41.9	4.6	
Whole corn	do	52.5	3.8	.001
Corn-gluten meal	do	41.8	5.4	
Soybean meal 1	do	61.1	3.7	.001
Soybean meal 2	do	68.5	3.3	
Soybean meal 1	Digestibility	83.9	3.5	.83
Soybean meal 2	do	84.3	2.7	

Table 3 shows that when the endogenous urinary nitrogen is corrected for body weight, a significant fall is noted between the first and second periods, but not when the correction is made for body surface. This is also shown to be true for the data from Mitchell's laboratory (*3*). A significant difference is shown between the mean biological value of whole-wheat and wheat-gluten proteins, between whole-corn and corn-gluten proteins, and between raw and heated soybean-meal proteins. However, no significant difference is shown in the digestibility of the two soybean meals.

The results obtained by the chemical method of Almquist et al. (*1*) are given in table 4. The digestibility determinations of the proteins in vitro do not agree very closely with the corrected digestibility obtained from the rat data. The corn proteins show a higher digestibility in vivo while whole-wheat, soybean, and tankage proteins tend to give better digestion in vitro. The differences between digestion of tankages and liver meal by pepsin HCl and on peptic-tryptic digestion show that a considerable amount of porphyrin-like material is present, and therefore there is a distinct error in the method employed by Almquist and associates for determining digestibility. The digestibility used in calculating the protein quality index was the value obtained in vitro which most nearly approached the digestibility in vivo. The explanation of this is given in the last column of table 4. The data indicate that no single method of enzymatic digestion is comparable with animal digestion.

TABLE 4.—*Results obtained for digestibility of proteins from various sources, expressed as percentage of total nitrogen, chemical (in vitro) means of digestion and determination being used*

Source of protein	Copper precipitable (A) [1]	Phosphotungstic acid precipitable (D) [1]	Undigested with pepsin (B) [1]	Undigested with pepsin neutralized after digestion	Undigested with pepsin and trypsin	Undigested residue as determined from rat data	Hot-water soluble (C)	Protein quality index	Method of digestion used for calculation
Dried whole egg	100.0	0.0	4.2		0.0	2.2	4.1	93.3	Pepsin.
Casein	95.7	.5	4.7		.1	.3	3.8	93.5	Trypsin.
Whole wheat	81.3	4.2	5.8		3.5	8.3	13.4	69.2	Pepsin.
Wheat gluten	84.0	8.3	.6		.2	.7	16.0	77.1	Do.
Whole corn	85.4	8.7	41.0		17.4	8.7	2.0	70.3	Trypsin.
Corn-gluten meal	94.0	2.3	10.8		5.9	3.3	1.8	87.9	Do.
Liver meal	86.9	5.9	11.0	15.3	11.6	11.7	11.5	73.1	Do.
Tankage	78.9	11.7	11.1	13.9	9.0	16.7	16.2	60.0	Pepsin, neutralized.
Soybean meal 1	95.4	.3	9.9		3.5	16.1	9.0	80.1	Pepsin.
Soybean meal 2	96.2	.4	11.3		3.7	15.7	6.5	81.2	Do.

[1] Protein quality index $= A-(B+0.6\ C)+0.4\ D$.

The data of tables 1 and 4 are compared in figure 4. There seems to be little correlation between the two methods. However, it will be noted that the natural foods, to which no protein material had been added or from which none had been subtracted, lie on a fairly straight line. Casein, corn gluten, and wheat gluten, which are more or less isolated proteins, give quite different results by the chemical method as compared with the nitrogen-retention method.

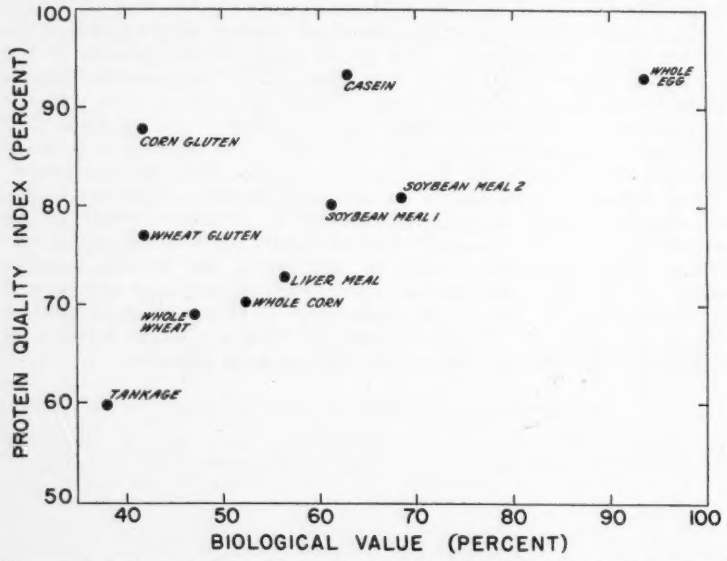

FIGURE 4.—Comparison of the protein quality index with the biological value for proteins from different sources. Data from tables 1 and 4.

SUMMARY

Nitrogen-balance studies were made with young rats, 10 food proteins being used. The values obtained (rounded) were: Whole egg 94, casein 63, whole wheat 47, wheat gluten 42, whole corn 52, corn-gluten meal 42, liver meal 56, meat and bone tankage 38, solvent-extracted soybean meal 61, and heated solvent-extracted soybean meal 68.

The same foods were used for the determination of the protein quality index by the Almquist chemical method.

The digestibility of the proteins was calculated from data obtained by the nitrogen-retention method. An attempt was made to compare these values with the digestibility determined by means of enzymes. The digestibility in vitro showed a poor comparison with the digestibility in vivo.

The protein quality index showed no comparison with the biological value by the nitrogen-retention method when used on isolated proteins. For natural foods the comparison is fair if the enzymic digestibility most similar to that in vivo is used for the calculation of the protein quality index.

Heated soybean meal was found to have a higher biological value than raw, but no difference was found in the digestibility of the raw and heated meals.

Correction of the endogenous urinary nitrogen according to body surface was found to give less variation than correction according to body weight. When the body surface correction was made, no difference was shown in endogenous urinary nitrogen between the first and second nitrogen-free periods.

The regression lines were plotted for the endogenous urinary nitrogen when calculated according to body weight and body surface area. The formula for the regression line for the body weight graph is $N=135.6W^{-0.469}$ and for the body surface graph, $N=5.61A^{0.069}$.

LITERATURE CITED

(1) Almquist, H. J., Stokstad, E. L. R., and Halbrook, E. R.
1935. Supplementary values of animal protein concentrates in chick rations. Jour. Nutr. 10: 193–211, illus.

(2) Ashworth, Ural S.
1935. Growth and development with special reference to domestic animals. XXXVI. Endogenous nitrogen and basal energy relationships during growth. Mo. Agr. Expt. Sta. Res. Bul. 223, 20 pp., illus.

(3) ———
1935. Growth and development with special reference to domestic animals. XXXVII. Interrelations between protein intake, endogenous nitrogen excretion, and biological value of protein. Mo. Agr. Expt. Sta. Res. Bul. 228, 14 pp., illus.

(4) Fisher, R. A.
1928. Statistical methods for research workers. Ed. 2, rev. and enl., 269 pp., illus. Edinburgh and London.

(5) Hawk, Philip B., and Oser, Bernard L.
1931. A modification of the Osborne and Mendel salt mixture. Science 74: 369.

(6) Lee, Milton O., and Clark, Elizabeth.
1929. Determination of the surface area of the white rat with its application to the expression of metabolic results. Amer. Jour. Physiol. 89: 24–33, illus.

(7) MASON, INEZ D., and PALMER, LEROY S.
1935. UTILIZATION OF GELATIN, CASEIN, AND ZEIN, BY ADULT RATS. Jour. Nutr. 9: 489–505.
(8) MITCHELL, H. H.
1924. A METHOD OF DETERMINING THE BIOLOGICAL VALUE OF PROTEIN. Jour. Biol. Chem. 58: 873–903.
(9) ——— and BEADLES, JESSIE R.
1937. THE NUTRITIVE VALUE OF THE PROTEINS OF NUTS IN COMPARISON WITH THE NUTRITIVE VALUE OF BEEF PROTEINS. Jour. Nutr. 14: 597–608.
(10) ——— and CARMAN, G. G.
1926. THE BIOLOGICAL VALUE OF THE NITROGEN OF MIXTURES OF PATENT WHITE FLOUR AND ANIMAL FOODS. Jour. Biol. Chem. 68: 183–215.
(11) SHEPPARD, W. F.
1902. NEW TABLES OF THE PROBABILITY INTEGRAL. Biometrika 2: [174]–190.
(12) SMUTS, D. B.
1935. THE RELATIONSHIP BETWEEN THE BASAL METABOLISM AND THE ENDOGENOUS NITROGEN METABOLISM, WITH PARTICULAR REFERENCE TO THE ESTIMATION OF THE MAINTENANCE REQUIREMENT OF PROTEIN. Jour. Nutr. 9: 403–433.

COMPARISON OF THE ACCURACY OF TWO METHODS OF ESTIMATING FINENESS OF WOOL FIBERS[1]

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INTRODUCTION

Diameter of wool fibers is one of the most important points to be considered in evaluating a fleece, either with respect to some phase of scientific work or from a commercial viewpoint. The method most commonly used for estimating the diameter is to compare fibers from a sample in question with standard fibers of known diameter or with a mental picture of such standards. Hardy and Wolf (*4*)[2] recently described a method in which enlarged cross sections of the fibers in question were compared with similarly enlarged fibers of known diameters which make up the set of standards. The object of the present study was to compare the accuracy of these two methods.

MATERIAL AND METHODS

One hundred samples of wool were selected from samples available in the laboratory. These were obtained from Rambouillet, Corriedale, and Columbia yearling ewes at the United States Sheep Experiment Station, Dubois, Idaho. The average diameter of each sample was determined by measuring the actual diameter of the fibers, up to a maximum of 100, in a given area of the cross section. In some of the coarser samples there were not 100 fibers in the area; in 17 samples there were only 50 fibers, and in 13 samples 90 fibers. The diameter of each fiber was obtained by taking 2 measurements at right angles to each other and obtaining the average of these 2 measurements. These determinations were made after completion of the scoring work on the 100 samples. Consequently, there was no information on actual diameters available to the judges at the time the scoring was done.

As a check on the actual diameters of the fibers, the diameters were estimated by the count method described by Hardy and Wolf (*4*). A correlation of 0.98 was found between diameters estimated in this manner and the direct measurements of fibers described.

For convenience, the two methods compared in this study are designated in this paper as method A, in which enlarged cross sections of fibers were compared with cross sections of known diameter, and method B, in which fibers were compared directly with those of known diameter.

In method A, a bundle of fibers from each sample was cross-sectioned by the method described by Hardy (*3*). The cross sections were photographed on positive 35-mm. film strips and projected to 1,000 magnifications for comparison with the standard, which consisted of a

[1] Received for publication November 3, 1939.

[2] Italic numbers in parentheses refer to Literature Cited, p. 349.

similar film having photographs of cross sections of known diameter magnified to the same extent. The various cross sections in the standard set were projected in succession adjacent to the image of the unknown until the observer decided which sample of known diameter the unknown most nearly approached. This method is described in detail by Hardy and Wolf (*4*). The standard set used contained the following nine wool grades, based on spinning counts: 80s, 70s, 64s, 60s, 56s, 52s, 48s, 44s, and 40s.

In method B, fibers from the samples were compared with fibers in a set of standard samples, which included the same nine grades as were used in method A. This set of samples was selected as being representative of the respective grades on the basis of both visual appearance and measurements of the diameter of cross sections.

The diameter of each sample was estimated by each method on 3 different days by 5 judges all of whom were experienced in the grading of wool. Thus 1,500 observations were made by method A, and the same number by method B. Statistical analysis of the data was made to determine the accuracy of each method. The statistical procedures are indicated in the discussion of each phase of the analysis. For convenience in analysis and in presentation, the grades arrived at in both methods were classified from 1 to 9, corresponding to the wool grades of 80s to 40s. The term "score" as used subsequently in this paper refers to the class numbers representing the grades into which the samples were classified by the judges.

EXPERIMENTAL RESULTS

DIFFERENCE IN SCORING LEVELS OF THE TWO METHODS

The placement of the samples by the five judges, in methods A and B, is given in table 1. The distribution of the samples based on actual measurements of cross sections is also given in table 1 for comparison with the scores given by the judges. The superiority of method A as evaluated by this procedure is obvious, 96 percent of the samples being placed in the correct class or deviating only 1 class from the correct one, whereas with method B only 44 percent were placed correctly or within 1 class of the correct one.

In the greater number of deviations from the actual values under method A the diameters of the fibers were underestimated, that is, they were estimated to be finer than they actually were; whereas in method B the diameters of the greater number were overestimated that is, estimated to be coarser than they were. Since the totals given in table 1 are based on 100 samples and are average placements of 5 judges, the numbers may be considered as percentages or probability placements.

Another method of showing the accuracy of the determinations by the use of methods A and B is given in figure 1. The frequency based on actual measurements is plotted against the class scale and is given by the solid line. The placement of samples according to method A follows closely that of the direct measurements, whereas the distribution by the use of method B is strikingly different and indicates that the judges, not being able to distinguish the relative diameters of the fibers, tended to place them toward the middle of the scale.

TABLE 1.—*Comparison of methods A and B in average placement of 100 wool samples by 5 judges* [1]

Class No.	Range in average diameter of fibers [2]	Samples in each class—													
		As found by actual measurements	Deviating from actual measurements by number of classes indicated (+ signifies overestimation; −, underestimation), as estimated by—												
			Method A					Method B							
			0	+1	−1	+2	−2	0	+1	−1	+2	−2	+3	−3	+4
	Microns	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
1	16–17.9	11	8	3							6		1		4
2	18–19.9	16	6	2	8			2	2		2		10		
3	20–21.9	27	14	4	9			3		2	19		2		1
4	22–23.9	21	10	2	9			1	14		5		1		
5	24–25.9	8	5	1	2			3	3				1		1
6	26–27.9	6	1		5				2	2	2				
7	28–29.9	4	1		2		1	2	2						
8	30–31.9	5			2		3	2	2			1			
9	32–37.9	2			2					2					
Total		100	45	12	39	0	4	13	25	6	34	1	15	0	6

[1] For description of methods, see p. 343 and 344.
[2] The range in diameter in each class was arrived at arbitrarily, the distribution curve being based on actual measurements of the ninth class, which is wider because of the few samples occurring in this group; therefore, the ranges differ somewhat from those given by Hardy and Wolf (4).

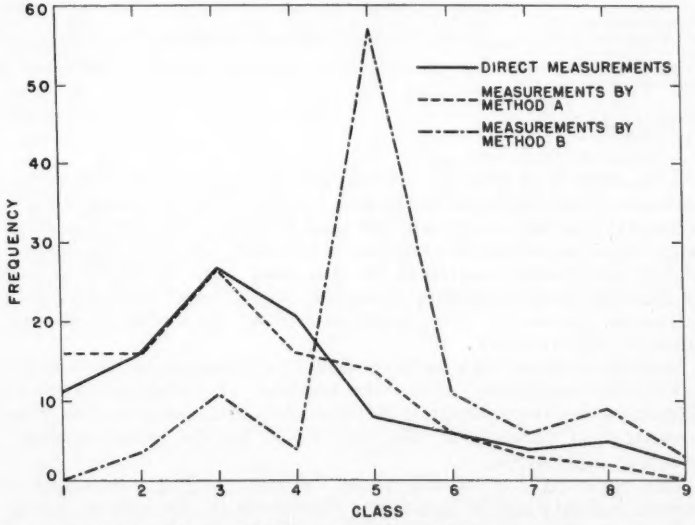

FIGURE 1.—Distributions of average diameters of 100 samples of wool, as determined by 3 different methods.

The frequencies obtained with methods A and B were correlated with the frequency obtained with the direct measurements. In method A, the correlation was 0.95; in method B, 0.77. These correlations are significantly different from a population in which the correlation is zero. In order to test the difference between the corre-

lations of 0.95 and 0.77, the method given by Fisher (*2*) was used. The probability of these values arising from the same population is less than 0.001. This finding is taken as statistical support to the conclusion that methods A and B are completely different in their placements of the samples in relation to the standard values.

SOURCES OF VARIANCE IN THE TWO METHODS

In order to evaluate the elements that contribute to the variability in the procedures, the data obtained by each method, A and B, were studied separately by using Fisher's analysis of variance (*2*). The mean squares calculated in that analysis were then transformed into percentages according to a plan first adopted by Lush [3] in a similar study, and later used in a study of two scoring methods employed in evaluating certain swine characters by Hetzer and Phillips (*5*). Table 2 gives the proportion of the variance due to factors which it is believed have an important influence on the variability of the scores. The method for testing significance was the *F* test given by Snedecor (*6*) for differences between mean squares as calculated in the analysis.

TABLE 2.—*Percentage of variance contributed by each source to the total variance in the scores by the 2 methods*

Source of variance	Method A	Method B	Source of variance	Method A	Method B
	Percent	*Percent*		*Percent*	*Percent*
Between samples	[1] 82.31	[1] 70.73	Judges×days	[2] 0.59	[2] 0.69
Between judges	[1] 4.76	[1] 4.55	Samples×judges×days	9.56	4.58
Between days	[1] .46	.03			
Judges×samples	2.53	[1] 13.02	Total	100.00	100.00
Days×samples	−.21	6.40			

[1] Highly significant ($p = <0.01$).
[2] Significant ($p = <0.05$).

The error term used in testing the significance was the source of variance listed as samples×judges×days. The percentages of variance owing to this source are included in table 2 to show the relative importance of causes of variation other than the first six listed. A part of the variance expressed by this error term evidently is caused by random error in scoring; however, other factors such as shifts in individual opinion of the judges in scoring may also be important causes of this variance.

In both methods the variance due to differences between samples was highly significant. Since the methods are designed to measure differences between samples, the one that demonstrates the greater percentage of variance in this item would be the better method for detecting differences.

The variance due to differences between judges was sufficiently large to indicate highly significant differences in the scoring levels of the judges. The amount of variation contributed by this source was about equal in the two methods.

Variance due to differences between days was small in both methods but is sufficiently large to be highly significant in method A because it enters into the scoring 500 times a day; therefore, the mean square becomes significantly large.

[3] LUSH, JAY L. REPEATABILITY OF SCORES MADE BY THE SAME MAN. [Unpublished.]

Of the double interactions, only one, the interaction of judges and samples in method B, was highly significant. Interactions of judges and days were significant in both methods, although in neither case is the variation large enough to be considered important.

CORRELATIONS BETWEEN SCORES

The extent to which the scores of the samples were likely to be the same on different days, the correspondence of the scores of the same judge on different days, and the extent to which the judges agreed in their scores for the same sample, are given by the intraclass correlations in table 3. These correlations are all highly significant. The correlations are similar in size for both methods, but in all cases those for method A are slightly higher, indicating a tendency for greater accuracy of method A as judged by these intraclass correlations.

TABLE 3.—*Intraclass correlation between the scores given each sample by the 2 methods*

Intraclass correlation	Method A	Method B
Between scores of samples on different days	0.98	0.90
Between scores of the same judge on different days	.87	.86
Between scores of the same sample by different judges	.93	.82

INDIVIDUAL-JUDGE PLACEMENTS

Since it is of interest to know how the individual judges placed the scores in the two methods, the data were grouped by judges, as given in table 4. The number of times each judge placed the sample correctly is given under each method. The plus and minus deviations also are given. The correlations (r) indicate the consistency of the judges in placing the samples. For example, judge No. 5 under method A placed the fewest samples correctly. However, the consistency with which he placed the samples is shown by the highest correlation, 0.84. Under method B the judges seemed to be consistent in their placement as indicated by the correlations, but the number of accurate placements was low, and the deviations were preponderately on the plus side, that is, the fibers were judged to be coarser than they actually were.

TABLE 4.—*Placement of scores by the individual judges, using the 2 methods*

Judge No.	Deviation from actual measurements by number of classes indicated (+ signifies overestimation; −, underestimation) as estimated by—																
	Method A								Method B								
	−3	−2	−1	0	+1	+2	+3	r	−2	−1	0	+1	+2	+3	+4	+5	r
	No.	No.	No.	No.	No.	No.	No.		No.	No.	No.	No.	No.	No.	No.	No.	
1	----	5	28	48	18	1	----	0.81	----	7	11	25	24	22	9	2	0.61
2	----	1	12	47	33	7	----	.81	3	6	13	30	30	11	4	3	.63
3	1	7	39	44	9	----	----	.82	2	8	27	27	25	10	1	----	.79
4	----	7	38	43	10	1	1	.81	2	8	6	24	22	24	12	2	.61
5	----	17	44	37	2	----	----	.84	1	----	15	27	25	24	6	2	.67

The general observations to be made from table 4 are that all judges were working within either method with approximately the same degree of success, and the relative accuracy of the two methods is not biased by individual judges.

DISCUSSION

The experimental findings indicate clearly that method A is more accurate in estimating wool-fiber diameter than is method B. With method A the judge not only is able to see the cross sections of fibers at 1,000 magnifications, but also he can view them without being confused by grease and dirt, crimp, and differences in light refraction. Furthermore, he is able to note and consider variability in the diameters of individual fibers. The psychological and physiological effects of these various factors on the wool scorer are discussed by Tänzer (*7*).

The fundamental basis of the visual judgment of the fineness of Wool is the Fechner-Weber psycho-physical law, as discussed by Barker (*1, pp. 141–151*). This law was stated by Fechner in 1860 as follows: "In order that the intensity of a sensation may increase in arithmetical progression the stimulus must increase in geometrical progression." His general formula is $I=C \log S$, where I is the sensation, S the stimulus, and C a constant. The failure of the judge to place the fineness of wool in the correct groupings or classes seems to indicate that the so-called law does not hold.

Method A requires much more equipment and time than method B, and in some types of work this expenditure would not be justified. However, it seems evident that method B cannot be expected to yield satisfactory data for experimental or other purposes in cases in which the detection of small differences in diameter is essential.

SUMMARY

A comparison is made of the accuracy of two methods for estimating the diameters of wool fibers. In method A the diameters of cross sections of 100 wool samples, after being projected to 1,000 diameters, were estimated by comparing with a standard set of known diameters enlarged to the same magnitude. In method B the diameters of wool fibers from the same 100 samples were estimated by comparing with a standard set of wool samples of known diameters. Five judges estimated the diameter of each of the 100 samples on three different days, making a total of 1,500 observations by each method. For comparison, the average diameters of the 100 samples were determined by actual measurements of the fibers in enlarged cross sections.

By method A, 45 of the 100 samples were placed correctly, based on the average scores for the judges, and only 4 deviated as much as 2 classes from the actual measurements. By method B only 13 samples were placed correctly, and 6 samples deviated 4 classes from the classes in which they were placed by direct measurements. Judges tended to underestimate diameter by method A and to overestimate by method B.

The distribution of the 100 fibers based on estimates of diameters by method A agrees closely with that based on actual measurements, whereas that obtained by method B is distinctly different.

An analysis of variance shows that 82.3 percent of the variance in the scores obtained by method A is due to differences among wool samples, whereas in method B only 70.7 percent is due to this source of variance.

Intraclass correlations between scores of samples on different days, between scores of the same judge on different days, and between scores of the same sample by different judges are all highly significant for both methods. In all cases the correlations for method A are higher but the differences are small. A study of the placements by individual judges indicates that all judges were working with about the same degree of accuracy within each method.

The findings indicate that the use of method A results in estimates of fiber diameters that are nearer the actual measurements, and that this method is more desirable than method B in all types of work in which the detection of small differences in fiber size is essential.

LITERATURE CITED

(1) BARKER, S. G.
1931. WOOL QUALITY: A STUDY OF THE INFLUENCE OF VARIOUS CONTRIBUTORY FACTORS, THEIR SIGNIFICANCE AND THE TECHNIQUE OF THEIR MEASUREMENTS. 333 pp., illus. London.

(2) FISHER, R. A.
1938. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 7, rev. and enl., 356 pp., illus. Edinburgh and London.

(3) HARDY, J. I.
1935. A PRACTICAL LABORATORY METHOD OF MAKING THIN CROSS SECTIONS OF FIBERS. U. S. Dept. Agr. Cir. 378, 11 pp., illus.

(4) ——— and WOLF, H. W.
1939. TWO RAPID METHODS FOR ESTIMATING FINENESS AND CROSS-SECTIONAL VARIABILITY OF WOOL. U. S. Dept. Agr. Cir. 543, 16 pp., illus.

(5) HETZER, H. O., and PHILLIPS, RALPH W.
1938. A STUDY OF TWO METHODS FOR SCORING CERTAIN CHARACTERS IN SWINE. Amer. Soc. Anim. Prod. Proc. 31: 141–146.

(6) SNEDECOR, GEORGE W.
1937. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. 341 pp., illus. Ames, Iowa.

(7) TÄNZER, ERNST.
1925. DIE SUBJEKTIVE WOLLBEURTEILUNG, KRITISIERT VOM STANDPUNKTE DER PSYCHOPHYSIOLOGISCHEN FORSCHUNG. Arch. f. Naturgesch. 91 A (Heft 9): 73–101.

CYTOLOGY OF PARTHENOGENESIS IN POA PRATENSIS[1]

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INTRODUCTION

Since Müntzing (*8*)[3] announced in 1932 that seed is set without gametic union (apomixis) in certain Swedish biotypes of *Poa pratensis* L. and *P. alpina* L., and with gametic union (amphimixis) in certain Swiss biotypes of *P. alpina*, several other investigators have reported evidence that apomixis occurs in *P. pratensis*. The cytological studies heretofore published, however, do not adequately explain apomixis in these species. The observations herein described pertain to the cytology of apomictic seed development in *P. pratensis*.

REVIEW OF LITERATURE

The studies of Müntzing (*8*) on Swedish biotypes of *Poa pratensis* showed that plants of different biotypes (or progeny from different plants) might have different somatic chromosome numbers, whereas plants of the same biotype (or the progeny from one plant, in one known instance) possess the same maternal chromosome number. He found also that plants with a common maternal origin were extremely uniform in morphological type and that the chromosome behavior in the meiotic divisions of the microsporocytes was frequently irregular although this irregularity had no apparent effect on fertility. Müntzing (*9*), in a study of twin seedlings, found that the two plants obtained from one seed usually had approximately the same number of chromosomes but that it was not uncommon for one member of such a pair to be approximately triploid in respect to the diploid chromosome number of its twin.

Nilsson (*10*, *11*, *12*) found that self-fertility might vary from 0 to 78.9 percent in different plants of *Poa pratensis* and that the fertility of open-pollinated plants might vary from 2.6 to 78.1 percent. He suggested that fertility in individual plants is affected by the environment and that in some plants sterility is due to defective sex organs. He concluded that the seeds of *P. pratensis* are set apomictically in nearly all cases. This species, however, presents a case of pseudogamy since pollination must be effected to induce seed development. Occasional intermediate forms appear, which Nilsson suggested result from gametic union.

[1] Received for publication July 1, 1939. The investigation herein described is a part of a project on the improvement of pasture grasses that is being conducted cooperatively between the Department of Agronomy, Wisconsin Agricultural Experiment Station, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] The author lost his life in the sinking of the S. S. *Athenia* on September 3 or 4, 1939, while on his return from the Seventh International Congress of Genetics, which met at Edinburgh, Scotland, August 23-29, 1939. In submitting the manuscript of the present paper the author asked that acknowledgment be made of the assistance of Arnold Lueck and Paul Ozanne, who aided in making the cytological preparations; of Prof. O. S. Aamodt, who contributed in many ways toward the completion of the work; and of Prof. C. E. Allen, who aided in the final preparation of the manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 360.

Åkerberg (*3*) also concluded that apomixis commonly occurs in *Poa pratensis*, although he has found four biotypes that regularly reproduce sexually. He found pollination necessary to initiate apomictic seed development; the pollen of *P. alpina* was nearly as effective in providing the stimulus to the development of the ovule of *P. pratensis* as was its own pollen. Fertilization is thought to occur occasionally in apomictic plants, since intermediate types appear. It is suggested from the chromosome number of one hybrid, that it arose from a fusion of an unreduced egg and a reduced male gamete (*1*). Another hybrid that Åkerberg obtained from pollinating unemasculated florets of *P. pratensis* with pollen from *P. alpina* suggested by its chromosome number that it arose from fertilization of a reduced egg by a reduced male gamete (*2*).

Cytological studies on the embryo-sac development have been made by Andersen (*4*) and Armstrong (*5*). Since Andersen's study was made prior to the suggestion that apomixis occurs in *Poa*, she naturally did not consider seed development from this point of view. She stated, however, that "fertilization was not observed by the author, although the slides were carefully examined for this detail."

Armstrong (*5*) found that plants from different strains of Canadian and European origin might have different chromosome numbers. All the strains that he studied gave evidence of the occurrence of meiosis in macrospore mother cells. He concluded, from the presence of paired chromosomes at diakinesis in macrospore mother cells and from the good germination of pollen, that fertilization occurs in two strains, Mammoth from Ontario and No. 994 from Aberystwyth. It is interesting to note that he found the plants of the Mammoth strain morphologically uniform.

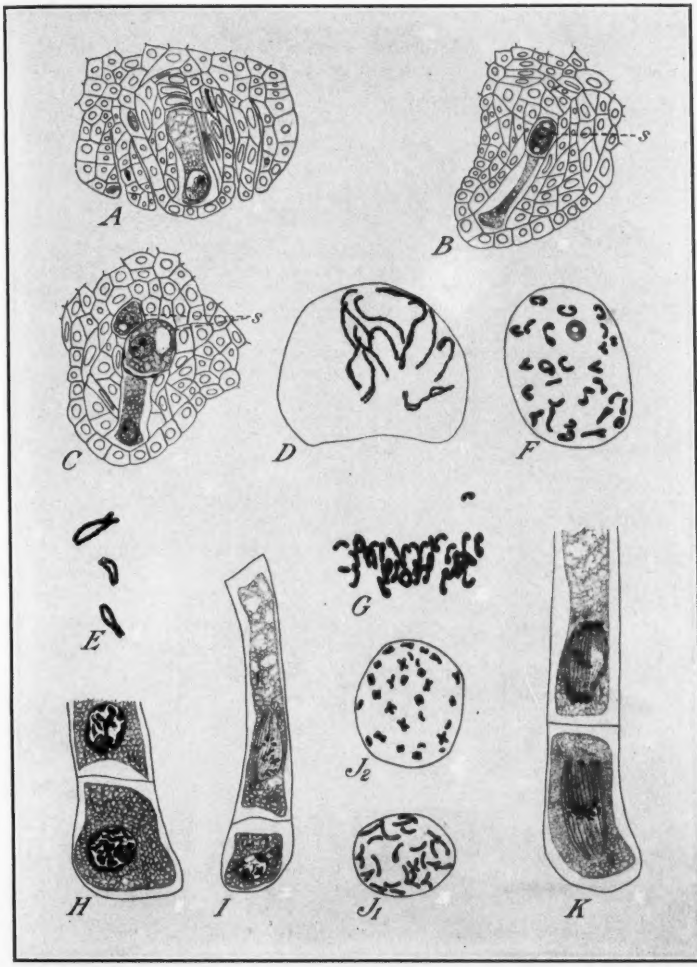
Other reported apomictic species in the Gramineae are: *Poa palustris* L. (*P. serotina* Ehrh.) (*7*); *P. alpigena* (E. Fries) Lindm., *P. glauca* Vahl., *P. arctica* R. Br. (*6*); *Calamagrostis obtusata* Trin. (*15*); and *Nardus stricta* L. (*14*). The complete cytological facts are not known for any of these species.

MATERIALS AND METHODS

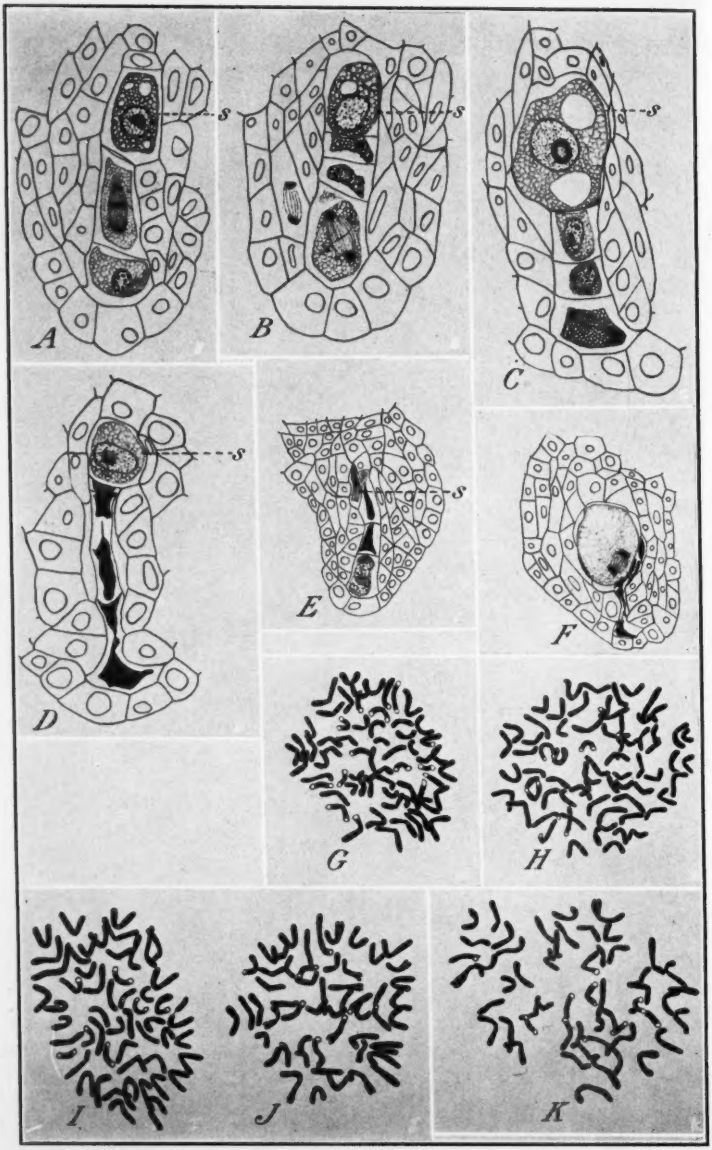
Seed of *Poa pratensis* collected from some of the oldest permanent pastures in Wisconsin was grown as single clones in the agronomy greenhouses and on the university farm at Madison, Wis. Most of the material for cytological study was collected from plants growing in the greenhouses during the past three winters, although some material was studied from collections made during summers in the field nursery.

In order to avoid confusion due to collecting material from plants differing among themselves in their method of reproduction, as many stages as possible were studied in material collected from the same clone. Altogether 5 clones of different biotypes have been studied in detail. In addition, material from 1 clone from each of 12 different biotypes was collected in the field nursery for a study of embryo development in the embryo-sac just before pollination.

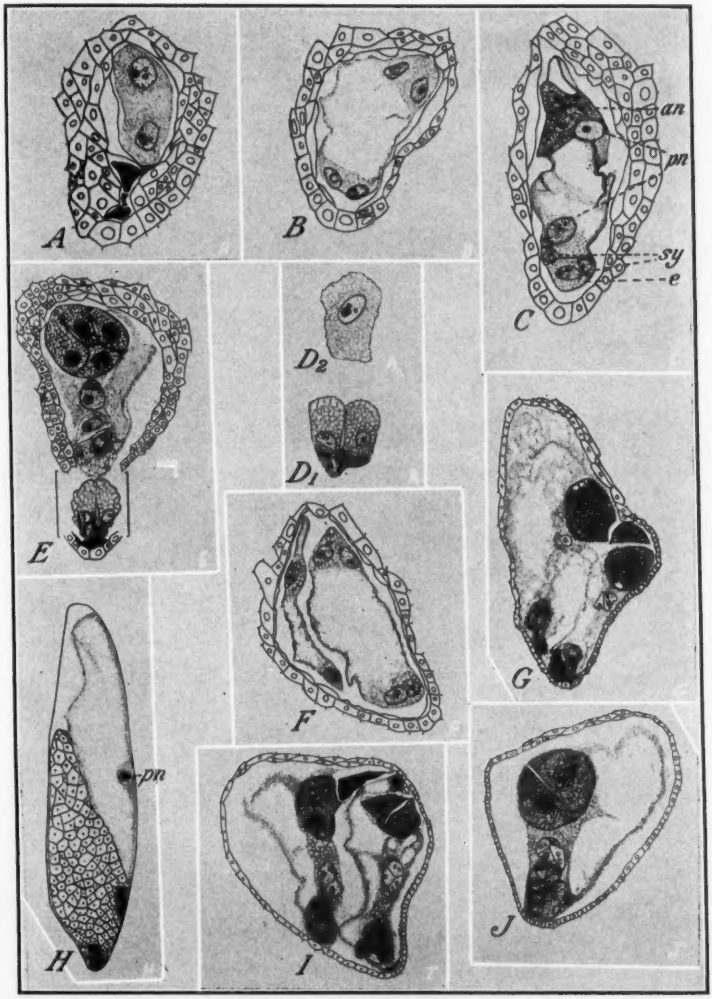
Individual florets were separated from the spikelets with the aid of a dissecting microscope, submerged for not more than 1 second in a solution of Carnoy's solution (6 parts absolute alcohol : 1 part glacial acetic acid : 3 parts chloroform), and then placed directly into Münt-



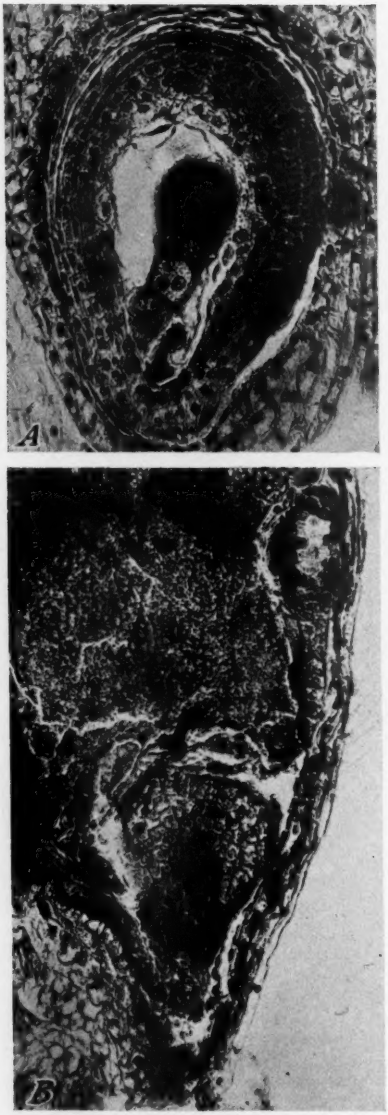
A, Young ovule with developing integuments and macrospore mother cell. × 218 *B*, Portion of nucellus, showing macrospore mother cell and differentiated somatic cell (*s*). × 218. *C*, Same as *B*, but showing macrospore mother cell and two differentiated somatic cells (*s*). × 218. *D*, Section through nucleus of macrospore mother cell illustrated in *A*, showing paired chromosome at diplotene. × 1,640 *E*, Portion of nucleus of a macrospore mother cell in early diakinesis. × 1,640. *F*, Nucleus of macrospore mother cell in late diakinesis. × 1,640. *G*, Metaphase of first meiotic divisions in macrospore mother cell (taken from plant shown in plate 2, *I* and *J*). × 1,640. *H*, Portion of daughter cells formed by first meiotic division; nuclei in interkinesis. × 629. *I*, Daughter cells; micropylar nucleus in interkinesis; chalazal nucleus in anaphase. × 462. J_1 and J_2, Second meiotic division; micropylar nucleus in early prophase, showing approximately 25 chromosomes (J_1); chalazal nucleus in late prophase, showing approximately 29 chromosomes (J_2). × 1,640. *K*, Portion of daughter cells, second meiotic division; micropylar nucleus in anaphase, chalazal nucleus in telophase. × 629. Drawings made with camera lucida at table level. All figures arranged with micropylar end of embryo sac toward bottom of page.



A, Portion of nucellus, showing two cells formed as result of first meiotic division; nucleus of micropylar cell in interkinesis and other in metaphase of second division; differentiated somatic cell (*s*). × 342. *B*, Same as *A*, but showing three cells formed as result of meiotic divisions (micropylar cell failed to complete second division); differentiated somatic cell (*s*). × 342. *C*, Same as *B*, but showing further enlargement of somatic cell (*s*). × 342. *D*, Same as *B*, but showing four disintegrated macrospores and differentiated somatic cell (*s*) separated from macrospores by vegetative cell. × 342. *E*, Same as *B*, but showing three cells in process of disintegration (micropylar cell failed to complete second division); early differentiation of somatic cell (*s*). × 180. *F*, Same as *B*; first division of nucleus in embryo sac and disintegrated macrospores. × 180. *G*, Metaphase; first division of nucleus in embryo sac, showing approximately 63 chromosomes. × 1,112. *H*, Metaphase; root tip of same plant as *G*, showing approximately 70 chromosomes. × 1,112. *I*, Same as *G* from another biotype, showing approximately 53 chromosomes. × 1,112. *J*, Metaphase; root tip from same plant as *I*, showing approximately 53 chromosomes. × 1,112. *K*, Metaphase; root tip from same plant as in plate 1, J_1 and J_2, showing approximately 54 chromosomes. × 1,112.



A, Portion of nucellus, showing two-nucleate embryo sac and three disintegrated macrospores. × 199. *B*, Same as *A*, showing four-nucleate embryo sac. × 199. *C*, Same as *A*, showing complete embryo sac, antipodals (*an*), polar nuclei (*pn*), synergids (*sy*), and egg (*e*). × 199. D_1, Synergids, and D_2, egg, from same embryo sac. × 199. *E*, Portion of nucellus, showing embryo sac with two-cell proembryo; two synergids, displaced by amount indicated to permit of their being drawn. × 141. *F*, Portion of nucellus with two developing embryo sacs: one binucleate, the other four-nucleate. × 199. *G*, Composite drawing from two sections, showing two complete embryo sacs each containing a proembryo. × 97. *H*, Composite drawing of later stage in seed development, showing two proembryos surrounded by endosperm of one embryo sac; two polar nuclei (*pn*) of second sac are unfused. × 26½. *I*, Composite drawing showing portion of nucellus and two embryo sacs, one of which possesses two polar nuclei; the other, five. × 97. *J*, Portion of nucellus with single embryo sac, showing four polar nuclei. × 97.



A, Photomicrograph of section of ovule collected just before pollination, showing proembryo with several cells. × 231. *B*, Photomicrograph of section of ovule collected after anthesis, showing two embryos. × 150.

zing's (*8*) modification of Navashin's solution or another modification composed of equal parts of two solutions, one of which consisted of 90 cc. of water, 10 cc. of glacial acetic acid, and 1½ gm. of chromic acid, and the other, of 40 cc. of formalin and 60 cc. of water. The air was removed with an aspirator. Fixation may be further enhanced by completely removing all floral parts and quickly submerging the pistils in the Carnoy fluid before placing them in the final fixing solution. This latter procedure was found especially useful when it was necessary to cut the ovules at particular angles in order to obtain polar views of the reduction divisions or of the first nuclear division in the embryo-sac mother cell. Root tips were fixed in Müntzing's solution. All material was left in the fixing fluid for from 18 to 24 hours and then washed in three or four changes of 70-percent alcohol (*13*). It was dehydrated in the usual manner, cleared in cedarwood oil, and embedded in paraffin. Root tips were cut at 10μ and stained in crystal violet iodine. Florets and pistils were cut from 10μ to 20μ, stained in Heidenhain's iron-alum haematoxylin, and destained in a saturated aqueous solution of picric acid.

OBSERVATIONS

The ovule in the basal florets of the spikelets, at the time the panicle begins to emerge from the sheath, consists of an outer and inner integument, each composed of two layers of cells, and a nucellus with a well-differentiated macrospore mother cell (pl. 1, *A*). A median section of an ovule at this stage invariably shows a single elongated, very conspicuous macrospore mother cell with the nucleus located usually near the micropylar end, or in some instances near the chalazal end. The cytoplasm is usually denser at the micropylar end than at the chalazal end of the cell.

In some instances at this early stage, one cell (pl. 1, *B*), or less frequently two cells (*C*), of the nucellus, near the chalazal end of the macrospore mother cell, differ conspicuously from the surrounding cells of the nucellus. The differentiated nucellar cell (or cells) is spherical, stains more darkly and, except for one or two characteristic vacuoles, contains denser cytoplasm than the surrounding cells of the nucellus. The significance of this cell will be discussed later.

The nucleus of the macrospore mother cell undergoes, so far as can be determined, a typical first meiotic division. Some tangential sections (pl. 1, *D*) clearly show paired chromosomes at diplotene. At later stages (*E*) the paired chromosomes appear to be held together by chiasmata. Stages in diakinesis (*F*) show that the chromosomes in most instances are associated in pairs, although univalents are frequently present. In one metaphase figure of the first reduction division (*G*) most of the chromosomes are associated in pairs, but there are three trivalent chains so arranged on the spindle that the two end chromosomes of the chain in each case will pass to the same pole; one chain of either three or four chromosomes is so arranged that the end chromosomes will pass to opposite poles; and three or four univalents are present. The first meiotic division forms two cells of which the one nearer the micropyle is usually the smaller (*I*). The nucleus of each daughter cell passes into interkinesis (*H*).

During the second meiotic division the chalazal cell precedes the micropylar cell in division. In one instance (pl. 1, *I*) the nucleus in the micropylar cell is in interkinesis when the one in the chalazal cell

is in anaphase; in another (J_1), the micropylar nucleus is in an early prophase, and the chalazal nucleus (J_2) is in a late prophase. J_1 and J_2 show that the number of chromosomes in each daughter nucleus is approximately one-half the somatic number of approximately 54 in the root tip of the same plant (pl. 2, *K*). In another instance (pl. 1, *K*), the nucleus of the micropylar cell is in anaphase and the nucleus of the chalazal cell in telophase. Univalents are frequently observed either passing precociously to the poles or remaining at the equator. In either case they frequently fail to be included in a daughter nucleus. Either daughter nucleus, then, may not receive a complete chromosome complement.

The second meiotic division in the chalazal cell regularly forms two haploid macrospores, both of which soon distintegrate. In most of the observed cases this second division is not completed in the micropylar cell because of the precocious disintegration of the cell (pl. 2, *B*, *C*, *E*). Consequently, only three cells are formed from the macrospore mother cell, although in one instance (*D*) four macrospores were observed. In all observed instances all the macrospores thus formed from the macrospore mother cell disintegrate and do not function in the development of the embryo sac.

A cell of the nucellus near the chalazal end of the macrospore mother cell has been described (p. 353) as being conspicuously different from the surrounding cells. In some instances (pl. 1, *B*, *C*) one cell or occasionally two cells are differentiated as early as the beginning of meiosis in the macrospore mother cell. Not infrequently, differentiation is not conspicuous until the meiotic divisions are completed (pl. 2, *E*). Plate 2, *A*, shows that of the two cells toward the micropyle, the nucleus of one is in interkinesis and the nucleus of the other is in metaphase, while the third cell, largest and inward, has been separately differentiated. In plate 2, *B* and *C*, the three micropylar cells that were derived from the macrospore mother cell are beginning to disintegrate. In the one nearest the micropyle, nuclear division has just been completed. The fourth and innermost cell has been differentiated separately. In another instance (pl. 2, *D*), four macrospores had been formed by two completed divisions and had completely collapsed. At the chalazal end of the macrospore row is a separately differentiated cell, separated from the row of disintegrated macrospores by a vegetative nucellar cell.

The differentiated nucellar cell just described is destined to function as the initial cell of the embryo sac (pl. 2, *F*). This conclusion is confirmed by the chromosome counts during the division of the primary nucleus of the embryo sac. In one instance (*G*) approximately 63 chromosomes were observed in this division. The chromosome number in cells of a root tip (*H*) of the same plant is approximately 70. In consequence of the high number of chromosomes and the resultant difficulty in distinguishing them, it is probable that the remainder of the diploid complement in the nucleus of the embryo sac was hidden from view. In another case, approximately 53 chromosomes were observed in nuclear division both in the initial cell of the embryo sac (*I*) and in a cell of a root tip (*J*) of the same plant. In several other cases a sufficiently accurate count of the chromosomes in the initial cell of the sac could be made to demonstrate the presence of the diploid number.

A two-nucleate embryo sac and three disintegrated macrospores are shown in plate 3, *A*, a four-nucleate sac in plate 3, *B*, and a completed eight-nucleate seven-cell sac in plate 3, *C*.

The mature embryo sac consists of three large, darkly staining antipodal cells at the chalazal end, a single central cell containing two polar nuclei, and the egg apparatus (egg and two synergids) at the micropylar end. The nuclei of the antipodal cells may divide and if cell division does not occur the cell in question remains binucleate. In some cases cell division follows to form ultimately five or six antipodal cells.

The synergids (pl. 3, D_1 and *E*) enlarge and become pear-shaped, with the apex of each projecting toward the micropyle. They lie side by side directly beside the egg. The cytoplasm in the basal portion of each synergid becomes very fibrous in appearance, constituting the filiform apparatus often described for synergids. A nucleus lies near the center of each synergid, and as the two cells disintegrate the nuclei maintain their structure longer than does the cytoplasm. In some instances it appears that the two synergids in the process of disintegration fuse into one darkly staining body.

The cytoplasm of the egg (pl. 3, D_2) becomes coarsely vacuolated; the nucleus lies near the center of the cell surrounded by denser cytoplasm. At the apical end the cytoplasm assumes regularly a more coarsely alveolar appearance, which seems to disappear when the cell divides.

The egg divides to form a proembryo in many of the first, second, or third florets of the spikelet before anthesis begins (pl. 3, *E*). The two synergids shown in *E* have been displaced by the amount indicated to permit of their being drawn. The proembryo shown in the photomicrograph (pl. 4, *A*) is larger than the one in the drawing (pl. 3, *E*), but the florets from which these figures were obtained were at approximately the same stage of flowering when fixed. In this instance the synergids have begun to disintegrate and have lost their identity. These observations have been made repeatedly in material fixed in the greenhouse under conditions that excluded the possibility of the presence of pollen in the air, and in material fixed in the field 1 or 2 days before the floral parts had opened to permit the exposure of the stigma. There seems no doubt that pollen is unnecessary to initiate development of the egg into an embryo. Since it has been shown that the nuclei of the embryo sac, including the egg nucleus, are all diploid, it is apparent that the functioning of the egg is not dependent on gametic union. The external stimulus to the egg, if one is necessary, is apparently exerted from within the embryo sac.

The two polar nuclei (pls. 3, *H;* 4, *A*) lie together in the dense cytoplasm of the embryo sac midway between the antipodals and the proembryo. Fusion of the polar nuclei is not completed until the proembryo consists of several cells, and occurs invariably after anthesis. Fusion is accomplished by dissolution of the adjacent membranes to form the primary endosperm nucleus, which later divides to form the endosperm nuclei. The cells of the endosperm, thus formed, would possess a $4n$ number of chromosomes in contrast to the $3n$ number usually found in sexually reproducing species.

It is not certain whether pollination is associated with endosperm development. Pistils examined a few hours after pollination show pollen grains germinating freely on the stigma and pollen tubes

growing down the style. In no case, however, have pollen tubes been observed in tissue of the ovary, or in the embryo sac. Florets that were emasculated and not polinated have in a few instances developed seeds, but it is possible that in these instances the stigma was accidentally pollinated during the act of emasculation. Florets that were open-pollinated in the greenhouse occasionally contained aborted ovules, and examination has shown proembryos but no endosperm. The two unfused polar endosperm nuclei were still frequently visible in such cases. The failure of the polar nuclei to fuse and to bring about the development of an endosperm may have been due to a failure of pollen tubes to stimulate endosperm development.

POLYEMBRYONY AND ATYPICAL EMBRYO SACS

Sections of young ovules not infrequently show two embryo sacs in course of development (pl. 3, *F*). The initial cells from which such sacs develop have not been observed in division, but it seems very probable from observations of ovules containing single embryo sacs that two sacs may arise independently from two differentiated nucellar cells, such as are sometimes present (pl. 1, *C*). Consequently, the nuclei in both embryo sacs would be expected to possess identical diploid chromosome complements. Andersen (*4*) concluded that polyembryony results from the functioning of two embryo sacs and that each embryo sac develops either from two macrospores in the same row or from two macrospores derived from separate macrospore mother cells.

One embryo sac, presumably that favored by position, may grow faster than its twin; the larger one develops in the usual manner, while the smaller develops irregularly and consequently may not produce a proembryo. If the development is not too irregular, each will produce a proembryo (pl. 3, *G*). In all observed instances in which two embryos were present there was evidence of the previous presence of two embryo sacs; for example, two sets of antipodals, or two pairs of polar nuclei, or both. The ovule illustrated in plate 3, *H*, contains two proembryos and a mass of endosperm tissue formed by division of the primary endosperm cell of one embryo sac. The previous presence of a second sac is evidenced by the two polar nuclei incompletely fused.

The embryo nearer the micropyle may be large and typical in shape; its twin embryo smaller, atypical, and located in an unusual position (pl. 4, *B*). Such instances, however, do not necessarily imply a difference in origin of the smaller embryo. These instances may be explained by the more rapid growth of one embryo and of its endosperm, in consequence of which the smaller embryo, in a less favorable position for growth, is crowded against the wall of the nucellar cavity.

There remains the possibility that one of the twins may have developed parthenogenetically from a haploid egg, in which case it would be expected to be weaker than the diploid twin. Müntzing (*9*) found one haploid plant which he suggested arose as a twin from the parthenogenetic development of a haploid cell. He found also in some instances that the two members of a pair, in the seedling stage, differ in size and vigor, perhaps in consequence of the more favorable position of the larger. He found the chromosome number of twin seedlings to be usually the same, but occasionally one was approximately triploid

with respect to its diploid twin; and if there was a difference in size, the triploid seedling, more frequently than the diploid, was the weaker of the two. Müntzing suggested that the triploid arose from the fertilization of a diploid egg.

The egg in each embryo sac (in case two sacs are present) frequently develops into a proembryo before pollination, and under such circumstances fertilization of the egg in either embryo sac obviously could not occur. The chromosome number would be the same in the two embryos (omitting the possibility of the development of a haploid embryo sac from a macrospore). If one or both eggs did not begin to develop until sufficient time had elapsed for the pollen tubes to reach the embryo sacs, one or both eggs might be fertilized. Müntzing (*9*) found both members of one set of twin seedlings to be approximately triploid. It seems reasonable to assume that the eggs in the weaker embryo sac might delay parthenogenetic development longer than those in the more vigorous one, and consequently provide opportunity for gametic union. Under such circumstances triploidy will be more frequently associated with the smaller of twin seedlings than with the larger.

There is no evidence that embryos originate by sporophytic budding from the nucellus.

Embryo sacs, when occurring either singly or doubly, occasionally contain more than the eight nuclei. In some instances an apparently functional embryo sac, in addition to the antipodals, two-nucleate primary endosperm cell, and egg apparatus, consists of single cells or nuclei lying in the cytoplasm of the nuclear cavity. It is probable that the condition might become too atypical for an embryo sac to function.

More than two polar nuclei are occasionally seen in process of fusion. In one instance (pl. 3, *J*) there were four polar nuclei; and in another (pl. 3, *I*), there were five polar nuclei in one embryo sac and two in a companion sac. The origin of these additional nuclei or cells is not known, but it seems probable that they result from nuclear divisions during embryo-sac development. It seems improbable that any of the nuclei shown fusing with the two polar nuclei came from a pollen tube.

DISCUSSION

The evidence presented by Müntzing (*8*), Nilsson (*12*), and Åkerberg (*3*) indicates that gametic union does not regularly occur in Swedish biotypes of *Poa pratensis*. According to Åkerberg and Nilsson, however, pollination is necessary to initiate seed development, and in a few cases they found evidence that pollination was followed by a union of male and female gametes. The chromosome number of one hybrid plant suggests that it arose from the union of an unreduced egg and a reduced male gamete (*1*). Müntzing (*9*) found that occasionally one member of a twin pair was approximately triploid and suggested that in such a case a diploid egg was fertilized by a haploid male gamete. In two instances the egg was thought to have been haploid. In one of these instances the egg was presumably fertilized by a haploid male gamete of *P. alpina* (*2*), while in the other the haploid egg presumably developed parthenogenetically (*9*). Åkerberg (*3*) found four biotypes that appeared to reproduce regularly by gametic union.

The cytological observations on *Poa pratensis* herein described show that in the biotypes studied the single macrospore mother cell underwent meiosis in the usual manner and formed haploid macrospores (usually three, since the second meiotic division was often incomplete). In all the observed instances the three or four macrospores so formed disintegrated. Andersen (*4*) reported, without presenting evidence, that the macrospore mother cell formed a row of four macrospores and that usually the chalazal macrospore formed the embryo sac although in several observed ovules the micropylar macrospore functioned. Armstrong (*5*) observed meiosis and concluded that one of the macrospores thus formed develops a haploid embryo sac and that meiosis ipso facto implies sexual reproduction.

The initial cell of the embryo sac is differentiated from among the vegetative cells of the nucellus while the meiotic divisions are under way, and develops into a typical eight-nucleate, seven-cell embryo sac. Consequently the nuclei of the embryo sac, including the egg nucleus, possess the same diploid chromosome complement as do the nuclei of the maternal parent. The egg is capable, consequently, of developing into an embryo without restoration of the diploid chromosome number by fertilization. Under these circumstances all the plants grown from seeds collected from a single plant possess the same diploid complement of chromosomes, and obviously are, therefore, similar in morphological type. Müntzing (*8*) has found that 10 plants examined in a progeny from a single plant all had the same maternal chromosome number. Observations made by the author indicate that all the plants (with few possible exceptions) grown from seeds collected from a single plant are alike in growth form and habit. Since the embryo sac develops from a vegetative cell of the nucellus without meiosis, its origin may be considered an instance of apospory. The embryo develops from the egg by parthenogenesis.

Cytological observations have not confirmed the conclusions of Åkerberg (*1*) and Nilsson (*12*) that pollination is necessary to induce seed development. These investigators based their conclusions on the lack of seed setting in emasculated florets, and obviously were not able to separate the two phases of the development of a seed, i. e., embryo development and endosperm development. The embryo of *Poa pratensis* develops in many instances before pollination, but since endosperm development does not begin until after pollination the effect of pollen or pollen tubes on endosperm development is not easy to determine. A comparatively few florets have been emasculated by the author, and some of these have produced seeds. In the emasculation the floret may have been injured or the pistil unintentionally pollinated. According to Åkerberg (*1*) and Nilsson (*12*), the environment also may affect seed development. Consequently, negative results might be due to any of several causes. There is some evidence to indicate that seed abortion is due to the lack of endosperm development, and it may be that in such instances pollen failed to germinate on the stigma.

The egg frequently begins development before pollination is effected, but occasionally development does not begin until after pollination. Since pollen tubes have been observed in the style, although not in the embryo sac, it is possible that occasionally one reached the embryo

sac before parthenogenetic development began and that a diploid egg was then fertilized. Circumstances such as this may lead to the production of an occasional hybrid as reported by Åkerberg (*1*), and of triploid twin plants such as were found by Müntzing (*9*). Chromosome disjunction during meiosis in the microsporocytes is extremely irregular, and in many instances chromosomes are lost from the daughter nuclei. It is not known whether chromosome-deficient pollen grains germinate on the stigma, although a deficiency appears to have no effect upon the development of pollen grains. If a gamete possessing a deviating chromosome number effects fertilization, the resulting zygote will be only approximately triploid. Müntzing (*9*) is not certain of the exact chromosome number in twin plants of *Poa pratensis*, but he suggests that the number is only approximately triploid. Once the hybrid is produced, the newly combined chromosome complements, either euploid or aneuploid, may be transmitted to future generations by parthenogenesis. Diploid parthenogenesis and occasional fertilization of the egg by a sperm possessing a varied chromosome number can produce the singular characteristics of *P. pratensis:* (1) Extreme polymorphism and varying euploid and aneuploid chromosome numbers of different biotypes and (2) constant morphological type and chromosome number in plants of the same biotype.

SUMMARY

Median sections through the micropylar region of young ovules of *Poa pratensis* invariably show a single, elongated, very conspicuous macrospore mother cell. The nucleus of this cell undergoes meiosis, and haploid macrospores are formed—usually three, since frequently the micropylar cell does not complete the second meiotic division. In observed instances, all the macrospores subsequently disintegrate.

The embryo sac develops, without meiosis, from a cell of the nucellus, which is located near the chalazal end of the macrospore mother cell. The typical mature embryo sac consists of three antipodal cells, a primary endosperm cell containing two nucelli, and the egg apparatus (egg and two synergids).

The diploid egg develops into a proembryo by parthenogenesis; the development begins frequently before pollination. (Possible exceptions are noted in the text.) Since endosperm development was not observed to begin until after pollination, it may be that pollination or the growth of pollen tubes in stylar tissue is necessary for endosperm development and consequently for seed development. Pollen tubes have not been observed in the embryo sac.

Diploid parthenogenesis and occasional fertilization of the egg by a sperm possessing a varied chromosome number can produce the singular characteristics of *Poa pratensis:* (1) Extreme polymorphism and varying euploid and aneuploid chromosome numbers between different biotypes and (2) constant morphological type and chromosome number in plants of the same biotype.

Two embryos are occasionally produced by the functioning of two embryo sacs. Each embryo sac appears to develop independently from separate somatic cells of the nucellus. There is no evidence that embryos arise by sporophytic budding from tne nucellus.

LITERATURE CITED

(1) Åkerberg, Erik.
1936. studien über die samenbildung bei poa pratensis l. Bot. Notiser 1936: [213]–280. [English summary, pp. 268–269.]

(2) ———
1936. bastard mellan poa pratensis l. × p. alpina l. artificiellt framställd. Bot. Notiser 1936: 563–566.

(3) ———
1938. seed production of the poa species. Svensk Frötidning 7: 79–83, illus. [Translation by R. Peter Jones in Imp. Bur. Pastures and Forage Crops, Herbage Rev. 6: 228–233, illus.]

(4) Andersen, Alice M.
1927. development of the female gametophyte and caryopsis of poa pratensis and poa compressa. Jour. Agr. Res. 34: 1001–1018, illus.

(5) Armstrong, J. M.
1937. a cytological study of the genus poa l. Canad. Jour. Res. (C) 15: 281–297, illus.

(6) Flovik, Karl.
1938. cytological studies of arctic grasses. Hereditas 24: [265]–376, illus.

(7) Kiellander, C. L.
1935. apomixis bei poa serotina. (vorläufige mitteilung). Bot. Notiser 1935: 87–95.

(8) Müntzing, Arne.
1932. apomictic and sexual seed formation in poa. Hereditas 17: 131–154, illus.

(9) ———
1937. polyploidy from twin seedlings. Cytologia Fujii Jubilaei Volumen, June 1937: 211–227, illus.

(10) Nilsson, Fredrik.
1933. själv- och korsbefruktning i rödsvingel (festuca rubra l.), ängsgröe (poa pratensis l.) och ängskavle (alopecurus pratensis l.). (With an English summary.) Bot. Notiser 1933: 206–230. [In Swedish. English summary, pp. 221–223.]

(11) ———
1934. studies in fertility and inbreeding in some herbage grasses. Hereditas 19: 1–162.

(12) ———
1937. frösättningen hos ängsgröe, poa pratensis. Bot. Notiser 1937: 85–109. [In Swedish. English summary, pp. 106–108.]

(13) Randolph, L. F.
1935. a new fixing fluid and a revised schedule for the paraffin method in plant cytology. Stain Technol. 10: 95–96.

(14) Schnarf, Karl.
1929. embryologie der angiospermen. *In* Linsbauer, K., Handbuch der Pflanzenanatomie . . . , Abt. 2, t. 2, 689 pp., illus. Berlin.

(15) Stenar, Helge.
1933. parthenogenesis in der gattung calamagrostis embryobildung bei calamagrostis ortusata trin. und calamagrostis purpurea trin. Arkiv. för Bot. 25A, No. 6, 8 pp., illus.

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